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FEB 20 1950

Volume 40 *Number 2*

133

Phytopathology

AN INTERNATIONAL JOURNAL

February 1950

Official Organ of

The American Phytopathological Society

$7.75 per year, Canada $7.50 per year, United States and dependencies $8.00 per year, elsewhere

Office of Publication

Corner North Queen Street and McGovern Avenue, Lancaster, Pennsylvania

Entered at the postoffice in Lancaster, Pennsylvania, as second-class matter. Acceptance for mailing at the special rate of postage provided for in paragraph 4, sec. 538, P. L. and R. Authorized May 12, 1921.

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HANS WILHELM WOLLENWEBER, 1879–1949

OTTO A. REINKING

The death of Professor, Dr. Phil. Hans W. Wollenweber on February 3, 1949, of a heart ailment at the home of his daughter, Mrs. Lore Schrider, in Silver Spring, Maryland, marked the passing of one of the world's outstanding mycologists. Dr. Wollenweber had come to the United States in September, 1948, from Germany, where he had retired from the Biologische Zentralanstalt at Berlin-Dahlem, to become an American citizen and to continue his research work in this country.

His outstanding work was the classification and relationship of Ascomycetes with the Fungi Imperfecti, notably the genus Fusarium. An internationalist, extremely broad-minded, he drew many foreign scientists, including French, Americans, Japanese, and workers from South Africa and South America, to his laboratory in Berlin-Dahlem. Dr. Wollenweber gave much of his time and energy to help all who came to him. Through these many contacts, along with the training they received and their resultant publications, and through his own research and publications, he greatly advanced his favorite branch of science.

He was born on September 9, 1879, on a farm in Stadthagen (Schaumburg Lippe), Germany. His father, Georg Wollenweber, was the owner of a nursery and also specialized in park planning and landscape gardening. Hans, along with his two sisters, spent his childhood on his parents' estate. His mother, Laura, a beautiful woman from Saxony, was interested in outdoor life and introduced ice skating for ladies in the small town of Stadthagen. Thus it came about that Hans Wollenweber's childhood was influenced by outdoor plant life and sport. Encouraged by his mother, as a small boy he assembled a collection of butterflies and other insects, and mushrooms, berries, and stones. At this early age, happily interested in science, he was called the "Forscher," or investigator, by his family. From six to sixteen years of age he attended the "Prorealgymnasium" in Stadthagen and then went for two years to the "Realgymnasium" in Bückeburg where he completed the "Obersecunda" course.

From April 1, 1896, until March 3, 1899, Hans Wollenweber was an apprentice in his father's nursery. He continued his studies in horticulture from April 1, 1899, through March 30, 1901, at the "Königliche Gärtnerlehranstalt" in Wildpark, Potsdam. These studies were interrupted during 1901 and 1902 by one year of compulsory military training in the 77th infantry regiment of Hanover. Being at that time and throughout his life averse to anything military, he was only too well satisfied to get back into his horticultural work, which he pursued for one year (from April 1, 1902, to March 3, 1903) as a garden technician with the city park director in Hanover. There he also undertook the task of landscaping the parks and surroundings of his home town, Stadthagen, which was not far distant. He

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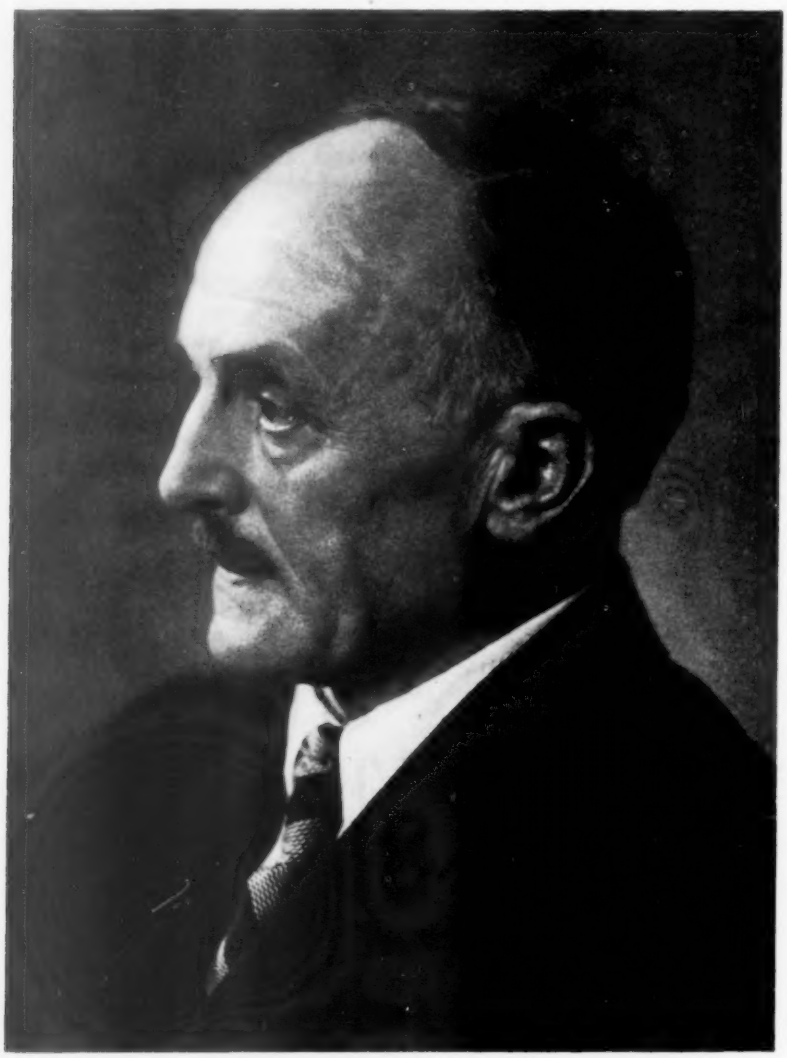
did such a splendid piece of work that the townspeople rewarded him with a gold watch, which he treasured all his life.

The practical application of his studies up to this time was not sufficient to satisfy his longing for more learning in the realm of science. He decided



HANS WILHELM WOLLENWEBER
1879–1949

to continue his undergraduate work and to go on with graduate studies in plant sciences. His undergraduate work was completed in September, 1904, at Leer in Ostfriesland. From there he entered college in Göttingen for botanical studies and finished at the University of Berlin. For his doctor-

ate he chose the investigation of the algal genus Haematococcus found in northern Europe and in Norway, where he spent his summers collecting and studying. While in Norway he studied under Professor N. Wille and other botanists, making valuable contacts that helped him greatly in later years. He fell in love with Norway, having learned the language, and whenever possible in later life he visited colleagues and went on field trips for study of fungus and plant life along some of his favorite fiords near Oslo. Dr. Wollenweber's prolific scientific writings began with the publication of his thesis on a preliminary study of the Haematococcus genus and a later paper on this genus. He received his doctor's degree in 1908 from the University of Berlin where he studied, among others, under Dr. Otto Appel.

After graduation from the University of Berlin, he accepted an appointment on January 1, 1908, as assistant to Dr. Behrens and Dr. Appel in the Botanical Laboratory of the Kaiserlichen Biologische Anstalt für Land- und Forst-wirtschaft in Berlin-Dahlem. It was during these years of investigations from 1908 to 1910 that he laid the basis for his monumental studies of the genus Fusarium and the Hyphomycetes. He was placed on a study of potato fusaria and soon found that systematic investigations were needed to straighten out this genus and its relationship to perfect stages. These investigations led to publications on potato fusaria and the Hyphomycetes, and finally to the joint monograph with Dr. Otto Appel entitled "Grundlagen einer Monographie der Gattung Fusarium." During the interval at Berlin-Dahlem, other papers on fusaria of potatoes and on the genus Fusarium were published.

In 1910, Dr. Wollenweber returned to Norway for a short time to continue his studies with Professor Wille. His friendship with Norwegian scientists and interest in their work were shown in two later publications, one on the writings of Professor N. Wille and the other in 1930, a translation of Professor Emil Korsmo's book "Unkräuter im Ackerbau der Neuzeit," on weeds, agriculture, and botany in Norway. These works demonstrated his broad-mindedness and his ability as a linguist. The basic studies on Fusarium made in Berlin-Dahlem led, in 1911, to an offer through Dr. W. A. Orton for continued studies of fusaria on potatoes and other plants with the United States Department of Agriculture in Washington, D. C.

On October 25, 1911, Dr. Wollenweber married Frieda Hespe, daughter of a dry-goods store owner in Bückeburg, Germany, a town near Dr. Wollenweber's birthplace, where he completed his early schooling. He and his wife left immediately for the United States. His new work as Expert, Assistant Pathologist, made it possible for him to continue his studies on Fusarium and to coordinate the American studies of pathogenic fusaria with the basic studies started in Europe. While in the United States he traveled widely through the potato areas there and in Canada. A number of fundamental manuscripts on his fusaria studies during his three-years' stay in America were published. These further clarified the confused Fusarium picture of

plant pathogens. "Studies on the Fusarium Problem" included the taxonomy and pathology of the imperfect and perfect stages. The publication on Ramularia, Mycosphaerella, Nectria, and Calonectria clarified the relationship of these perfect stages with cylindrical and sickle-shape conidium forms, and the article on identification of species of Fusarium occurring on the sweet potato classified various Fusarium species along with their perfect stages. His identification of fusaria on various hosts for other workers in the Department led to the publication and clarification of other Fusarium plant pathogens.

Just before the outbreak of World War I, Dr. Wollenweber was sent to Europe by the Quarantine Board of the United States Department of Agriculture to correlate further the American potato diseases with those found in Holland, Belgium, Denmark, Switzerland, and Italy. Before leaving the United States, he and his family had become real Americans and planned to take out citizenship papers; his first son was born on August 27, 1912, in Washington, D. C. The European trip was interrupted by the outbreak of war, and while in Germany in December, 1914, being a German citizen, he was drafted into the German Army with the rank of Feldwebel and stationed at Cologne. On October 1, 1918, he was discharged from active duty because of a heart ailment and was transferred as pathologist to the newly established Forschungsinstitut für Kartoffelbau in Berlin-Steglitz. In 1924 this institute was merged with the Biologische Reichsanstalt in Berlin-Dahlem. He was placed in charge of the mycological work there and rose to the position of Regierungsrat in 1927 and, in 1932, to that of Oberregierungsrat. In 1946 he was promoted to Professor and Division Head in the Institute and continued in this capacity until 1948 when he retired.

During these years his productive work continued with such outstanding monographs on fusaria as the "Fusarium Monographie, Fungi parasitici et saprophytici," published in 1931, with a second part in 1943. These studies completed his systematic investigations with fusaria. Starting in 1917, and from time to time thereafter, appeared numbers of his "Fusaria autographice delineata," including 1200 plates of drawings and notes of Fusarium and other fungi prepared from herbarium specimens and pure cultures. Synonym notes and descriptions along with drawings in this tremendous work were the basis for much of his Fusarium monograph and the simplification of the genus, as well as for systematic work with other fungi. His entire card catalogue, giving detailed accounts and drawings, included over 8000 fungi he had studied.

Dr. Wollenweber's interest was not confined to the genus Fusarium; he made a systematic study of members of the Discomycetes, Pyrenomycetes, and Hyphomycetes as well. He is noted for his mycological studies published in Band II and III der 5 Auflage des Sorauer, Handbuch der Pflanzenkrankheiten. He even delved into the realms of zoology and nematology, and described new Chlamydomonas species and potato nematodes. Studies toward a systematic classification of Actinomycetes were

started. With his fellow scientist in Germany, Dr. H. Hochapfel, an extensive series of papers on investigations of parasitic and saprophytic fungi was published. Detailed descriptions and classifications of the various fungi, along with their fruit-rotting ability, were presented for the following genera of fungi: Phomopsis, Dendrophoma, Phoma, Ascochyta, Monochaetia, Pestalotia, Fusarium, Cylindrocarpon, Coniothyrium, Diplodia, Vermicularia, Colletotrichum, Gloeosporium, and Glomerella.

In addition to the systematic work with various fungus groups, Dr. Wollenweber conducted investigations and published on diseases of potatoes and tomatoes and their control, Dutch elm disease, fruit blight of quince, diseases of flax, vascular wilt diseases of plants, needle cast of Douglas fir and the cause of dying of pine trees, tree galls, cereal fusaria, Katjang root rot, and wood-preserving fungicides.

Between World Wars I and II, three trips were made to the United States. The first trip in 1924 was very important to the writer for he was instrumental in bringing Dr. Wollenweber over to help in a study of a large collection of tropical fusaria, collected in Central America during an investigation of the Panama wilt of bananas. Through Dr. W. A. Orton a plan for an American conference on Fusarium was organized. The conference was held at the University of Wisconsin on invitation of Dr. L. R. Jones. Present at the conference, other than Dr. Wollenweber, were Dr. Otto A. Reinking, Dr. C. D. Sherbakoff, Miss Helen Johann, and Mrs. Alice A. Bailey.

The purpose of the conference was to make a joint study of Fusarium classification. Collections were on hand for special study, comparison, and identification from Europe, the United States, and Central America, the major part of the time being devoted to the tropical collection. Species from all sections of the genus Fusarium, including important borderline strains, facilitated arrival at a uniform taxonomy of the group. The conference resulted in the publication of a paper on "Fundamentals for Taxonomic Studies of Fusarium" by all members. Later, papers were published on newly described Fusarium species by Dr. Wollenweber and the writer, as well as a monograph on tropical fusaria by the writer and Dr. Wollenweber.

Not only did the conference result in the formulation of a uniform taxonomy and better understanding of the principles of Fusarium classification, but it terminated in a better understanding between fellow scientists, and lifelong friendship with Dr. Wollenweber whose wonderful character and simplicity were enjoyed by all who came in contact with him. The conference was also the beginning of further mutual studies on Fusarium and Cylindrocarpon species and various joint publications on tropical members of these groups. Between 1933 and 1935 the writer lived at Dr. Wollenweber's home in Berlin-Zehlendorf while continuing studies at the Biologische Reichsanstalt für Land- und Forst-wirtschaft. There, combined studies terminated in 1935 in the publication of the book "Die

Fusarien ihre Beschreibung, Schadwirkung und Bekämpfung," and a booklet, "Die Verbreitung der Fusarien in der Natur."

Dr. Wollenweber visited the United States again in 1926 as participant in the International Phytopathological Congress in Ithaca, N. Y., and again in 1938 when he traveled throughout the West and East, visiting scientists and various experiment stations enroute. A pleasant month was spent with him and his daughter and family at the cottage of the writer on Keuka Lake, not far from Geneva, N. Y. When war was declared against Poland Dr. Wollenweber's ticket on a Dutch liner was canceled and only after great difficulty was return passage secured via the Azores, Italy, and Switzerland, into Germany. There he continued with his scientific research and publications on the Fusarium group and various other groups of fungi during the entire war.

He was a member of the German Dendrologischen Gesellschaft and The American Phytopathological Society; and was one of the collaborators of the fifth revised edition, under the editorship of Dr. O. Appel, of Sorauer's Handbuch der Pflanzenkrankheiten. As Professor in the Landwirtschaftlichen Hochschule in Berlin, he trained a number of the younger phytopathologists now leaders in various German experiment stations.

Dr. Wollenweber was a true scientist interested almost wholly in a study of his fungi. He worked hard each day in his laboratory and then late into the night at home, classifying the investigations made during the day. Detailed drawings and notes were made of all fungi studied. His work was fundamental, including a study of all specimens and publications available concerned with the fungi under investigation. His excellent drawings and complete notes were assembled, completely catalogued, in volumes which he left in his study at his home. These, along with his publications, are now a monument to his life's work.

While it was difficult to get him away from his investigations, he found time to enjoy walks through the parks, especially the "Grunewald" near his home in Berlin-Zehlendorf, and to enjoy the company of visiting scientists and friends over a cup of coffee at various park restaurants. His hobbies were piano playing, hiking, and travel. He enjoyed week-end trips into the country and was a great hiker. Many a delightful trip with him in the countryside in various parts of Germany was enjoyed by the writer and visiting scientists. He was humble and unassuming, never forced himself at social or scientific gatherings, but was always one of those who kept the conversation going. His enjoyment of nature and his very normal human trait of liking a joke made him a pleasant traveling companion. Whenever he had an opportunity, he traveled to various parts of Germany, Holland, Italy, and the Scandinavian countries, where he always had a host of scientist friends.

In spite of his almost complete devotion to his work, he brought up a family, to whom he was deeply attached, of three sons and one daughter,

after the early death of his wife in 1927. The oldest son, who was wounded in the last war, is now a state forester in the German Government Forest Service. The second son is still a prisoner of war in Russia and the youngest son has not been heard from after being on the Russian front. His only daughter married an American and is now living with her family in Silver Spring, Maryland.

Dr. Wollenweber was averse to military training and practice, as opposed to his belief in good will toward all mankind. He was not interested in politics and never joined the Nazi party. Naturally, he was a loyal German subject, but at the same time he was enough of an internationalist to see the other man's side and even to plan on becoming an American citizen when he lived in the United States. The world, therefore, has lost not only an outstanding scientist in the mycological field, but a real humanitarian who will be held in esteem for a long time to come.

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NICOTIANA RUSTICA AS A SOURCE OF TOBACCO STREAK VIRUS[1]

STEPHEN DIACHUN AND W. D. VALLEAU

(Accepted for publication July 29, 1949)

In developing varieties of tobacco resistant to the streak virus it is desirable to know how to maintain readily a good source of the virus and how to inoculate plants rapidly and consistently. Johnson (6) reported that streak virus could be transmitted to young tobacco plants without much difficulty. Extracts from young leaves or young suckers of field plants showing early stages of the disease gave a fair percentage of infection, and "transfers from young greenhouse plants invariably yielded 100 per cent infection on Havana-seed tobacco by means of the rubbing method of inoculation." Berkeley and Phillips (1) reported more difficulty, but they also found that the juice transfer method gave a satisfactory percentage of transmission, provided the juice was extracted from newly formed necrotic tissue and rubbed on leaves of young rapidly growing plants on which fine carborundum was sprinkled. To transfer the virus from plants that had outgrown the necrotic phase of the disease they found it necessary to use patch grafting. They pointed out that at times the percentage of infection even with patch grafts was low.

In our experience, necrotic tobacco leaves in the early acute stage of infection have been a variable and unreliable source of virus, especially where inoculum of high virus titre was needed to inoculate relatively resistant plants (2, 9). Fulton also reported (4) that inoculum prepared from necrotic leaves or upper tolerant leaves has a low concentration of virus. He found that inoculum prepared from inoculated tobacco leaves (*i.e.*, leaves rubbed with the virus several days previously) is a good source of the virus, provided the inoculum is used immediately after preparation.

To find a better and more reliable source of the virus and to overcome the inconvenience of maintaining a continuous succession of tobacco plants in the acutely necrotic stage or in the local lesion stage of the disease, the chronically affected leaves of several species of *Nicotiana* were tested. Preliminary tests showed that at times inoculum from young tobacco leaves in the chronic stage of the disease produced more local lesions than inoculum from acutely necrotic leaves. Later it was found that inoculum prepared from young chronically diseased ("recovered") leaves of *N. rustica* usually produced more local lesions on rubbed leaves of Burley tobacco than did inoculum from acutely or chronically diseased Burley tobacco leaves. The local lesions produced on Burley leaves by inoculum from leaves of *N. rustica* chronically infected with the strain of the virus used were clear-cut, discrete spots and rings that could be counted (Fig. 1).

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

PROCEDURE

Inoculum was prepared by crushing leaves with a glass spatula, described by Samuel (7), in a small aluminum dish with 1½ times their weight of M/10 Na_2HPO_4 (approximately equal volumes). The juice was squeezed out through a double layer of cheesecloth and rubbed with a glass spatula on the upper surface of leaves of Burley tobacco dusted with 600-mesh carborundum powder. Usually the inoculum was rubbed on with approximately 16 strokes of the spatula. Usually each preparation was rubbed on one area of 10 leaves; up to 8 areas could be inoculated on each leaf; thus

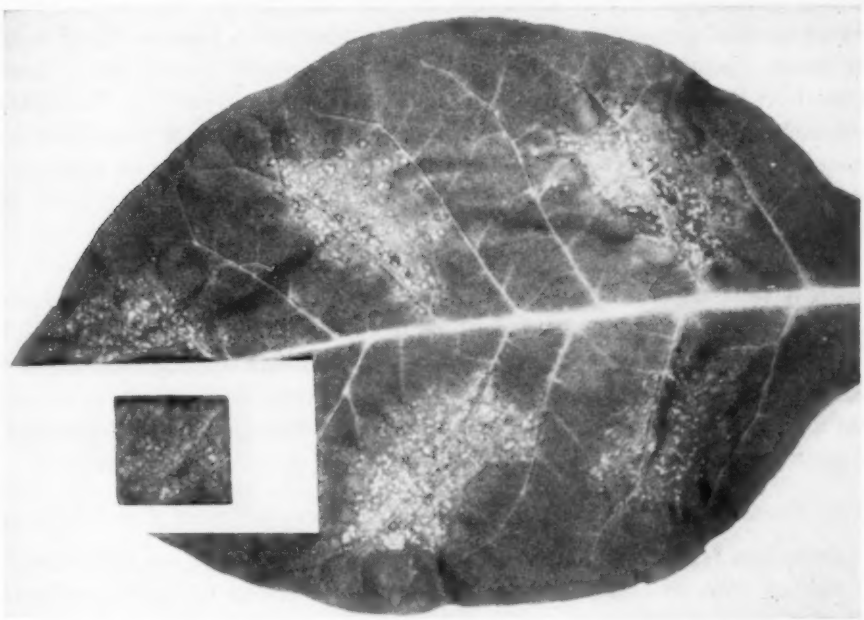

FIG. 1. Local lesions produced by streak virus on six rubbed areas of a Burley tobacco leaf; some lesions are confluent. Lesions are counted or estimated on an area of 16 cm^2.

by using 10 leaves as many as 8 preparations could be compared. The arrangement was often but not always randomized. Lesions were counted in an area of 16 cm^2. When more than 40 lesions were present in an inoculated area of 16 $cm.^2$ the number was estimated as being 50, 75, 100, 125, 150, 200, 250, or 300, by comparison with photographs and diagrams. The reported figures represent the total numbers of local lesions present in the 10 areas, unless stated otherwise.

EXPERIMENTAL RESULTS

The Effect of Carborundum and Na_2HPO_4

Berkeley and Phillips (1) reported more successful inoculations with streak virus when carborundum was used. Diachun (2) reported in abstract that M/10 Na_2HPO_4 increased the effectiveness of streak inoculum,

as had been previously reported for tobacco mosaic virus by Thornberry (8). The beneficial effect of carborundum and Na_2HPO_4 is shown in the following test:

Three young leaves (10 to 15 cm. long) of chronically diseased *Nicotiana rustica* were sliced into $\frac{1}{8}$-in. strips, mixed, and divided into two equal parts. One part was crushed with a glass spatula with an equal volume of Na_2HPO_4 and rubbed on one area of 10 tobacco leaves dusted with carborundum, and on 10 areas of these leaves not dusted with carborundum. Within 3 days there was a total of 1250 local lesions on the areas dusted with carborundum, and 71 lesions on the undusted areas. The second portion of the leaf strips was crushed with tap water and rubbed on 10 areas of the leaves dusted with carborundum. Only 10 lesions developed on these areas. At times the number of local lesions was small even when Na_2HPO_4 and carborundum were used. In two comparable tests the total number of lesions by the three types of inoculations was 96, 15, and 11 in one test; and 675, 8, and 75 in another.

Comparison of Inoculum from Nicotiana rustica and Tobacco

In 3 tests the total number of lesions produced on 10 carborundum-dusted tobacco leaf areas by inoculum from chronically affected leaves of *N. rustica* prepared with M/10 Na_2HPO_4 was 1210, 1350, and 1750. The number produced by inoculum from acutely necrotic Burley tobacco leaves was 5, 3, and 4, while the number produced by inoculum from chronically affected tobacco leaves was 24, 14, and 15.

Some Factors Tested for Their Influence on Production of Local Lesions

Inoculum from *N. rustica* did not always produce numerous local lesions on rubbed tobacco leaves. Several factors were tested to determine their effect on titre of inoculum.

Aging of inoculum. Inoculum prepared from chronically diseased leaves of *N. rustica* crushed moderately with M/10 Na_2HPO_4 did not deteriorate greatly after aging for several hours at room temperature. This means that possible differences in the speed with which inoculations were made at different times probably did not explain the variability observed among tests. In one test, for example, fresh inoculum produced 1725 lesions on 10 rubbed leaf areas; after standing 2 hr. in the greenhouse the inoculum produced 1125 lesions; and after standing 5 hr., 600 lesions. At another time fresh inoculum produced 1875 lesions; after standing 4 hr., 1800 lesions. In another test fresh inoculum produced 1025 lesions; after standing 5 hr., 750 lesions. At another time when the virus titre of the inoculum was not high, fresh inoculum produced 400 lesions; inoculum aged 3 hr. produced 325 lesions. Fulton recently reported (5) that aging for as short a time as 20 min. inactivated streak inoculum prepared from tobacco, and that rapid aging of the streak virus also occurs in extracts of infected

N. glutinosa and *N. rustica*. In the inocula used in the present study the relative resistance to inactivation by age might be explained on the basis of the manner in which the inoculum was prepared, as is discussed in a later section.

Age of leaves used for inoculum. In general, inoculum prepared from relatively young chronically diseased leaves of *N. rustica* was more active than inoculum from old leaves. In one test, for example, the fourth leaf below the bud, 8 cm. long, produced 2700 lesions on 10 rubbed leaf areas; the fifth leaf, 10 cm. long, 2750 lesions; the seventh leaf, 16 cm. long, 2750 lesions; the ninth leaf, 30 cm. long, 2380 lesions; the eleventh leaf, 33 cm. long, 980 lesions; the thirteenth leaf, 34 cm. long, 98 lesions; the sixteenth leaf, 37 cm. long, 570 lesions; and the eighteenth leaf, 37 cm. long, 38 lesions.

Age of inoculated leaves. Although very young rubbed leaves of Burley tobacco and very old leaves usually developed fewer lesions than leaves of intermediate age, especially on young plants, the effect was not great enough to explain the variability experienced in different lots of inoculum.

Amount of rubbing during inoculation. The number of local lesions was found to depend to some extent on the amount of rubbing. One stroke of the glass spatula dipped in inoculum did not produce as many lesions as did several strokes. In one test a single rub with the spatula produced 360 lesions on 8 leaf areas; 4 rubs, 705 lesions; 8 rubs, 850 lesions; 16 rubs, 1100 lesions; 32 rubs, 1250 lesions.

Vigor of plants used for inoculum. Although quantitative nutritional studies were not made, observation indicates that active, vigorously growing plants of *N. rustica* are a better source of the virus than old or slow-growing plants. This appears to be especially true during the winter months.

Degree of crushing in preparing inoculum. In early tests inoculum was prepared by crushing chronically diseased leaves of *N. rustica* with M/10 NA_2HPO_4 in a small aluminum cup with a glass spatula. The suggestion occurred that perhaps some of the variation observed was caused by different degrees of thoroughness of crushing, and that perhaps more thorough crushing might release more virus and produce good inoculum more consistently. Tests showed, on the contrary, that inoculum prepared in a mortar by thorough grinding with a pestle often produced fewer lesions than inoculum prepared by less thorough crushing of the leaves with a glass spatula. In 7 tests inoculum prepared by crushing with a glass spatula produced on 10 leaf areas 1375, 710, 1200, 528, 525, 760, and 1250 lesions; inoculum prepared by grinding in a mortar produced 565, 24, 54, 261, 440, 235, and 1 lesions. Moderate crushing in a mortar usually produced inoculum of intermediate titre. These results indicate that thorough grinding of infective tissue of *N. rustica* potentially high in active virus liberates some virus-inhibiting substance from the crushed cells.

Virus inhibitor in healthy leaves. Healthy juice was then tested for the presence of a streak virus inhibitor. In 13 tests the addition of an equal volume of healthy juice from tobacco leaves nearly always reduced the activity of the streak virus markedly. The number of lesions produced in these tests by inoculum prepared in the usual manner, as compared with the same inoculum mixed with an equal volume of healthy juice, was 1150 and 83, 2250 and 98, 520 and 7, 680 and 15, 1225 and 480, 400 and 21, 1350 and 825, 2000 and 29, 1300 and 25, 1850 and 49, 620 and 1, 1675 and 215, 1155 and 678. The reduction in the number of lesions was not due to dilution of the virus, for the addition of an equal volume of distilled water, tap water, or M/10 Na_2HPO_4 did not reduce the number of lesions. The figures show that healthy juice was not always equally effective as an inhibitor.

Similar tests showed that healthy juice had no inhibiting effect on the tobacco mosaic virus.

The presence of a streak virus inhibitor in healthy juice was reported in abstract form (3) and has been confirmed by Fulton (5). Fulton also reported that the inhibitor is more active and more consistently present in healthy juice aged 20 min.

Effect of Na_2SO_3. Fulton also reported (5) that reducing agents such as Na_2SO_3 incorporated in the inoculum at the time of preparation prevented aging of the inocula he used. In subsequent tests in this laboratory the addition of M/100 Na_2SO_3 to M/10 Na_2HPO_4 ensured the production of active inoculum consistently from chronically affected leaves of *N. rustica*. The presence of Na_2SO_3 seems to eliminate the harmful effect of thorough grinding. In three tests inoculum prepared by thorough grinding of young chronically diseased leaves of *N. rustica* in a mortar with M/10 Na_2HPO_4 produced 260, 440, and 235 lesions, whereas inoculum prepared with M/10 Na_2HPO_4 and M/100 Na_2SO_3 produced 2000, 1700, and 2250 lesions. Later tests showed that M/100 Na_2SO_3 without M/10 Na_2HPO_4 produced good inoculum. These results appear to be in harmony with Fulton's report that reducing agents, including M/100 Na_2SO_3, eliminate the streak-virus-inactivating substance present in extracts of tobacco leaves.

DISCUSSION

The streak virus is relatively difficult to transmit mechanically, especially to moderately resistant species and hybrids of *Nicotiana*, unless special measures are taken. Inoculum prepared at different times may differ in the number of local lesions produced on rubbed tobacco leaves. It seems that the concentration of streak virus is different in different hosts, such as tobacco and *N. rustica*, and perhaps in different stages of infection; and also that the virus is unstable or inactive under certain conditions. Even when inoculum is prepared from chronically diseased leaves of *N. rustica* that have a high content of the virus, local lesions are not numerous unless the virus is extracted in the presence of Na_2HPO_4 or Na_2SO_3. The effect

of these chemicals is not understood; they may serve to disperse the virus particles or to inactivate some inhibiting substance present in the plant sap. The action of an inhibitor is suggested by the fact that 1) thorough grinding of chronically infected leaves of *N. rustica* with M/10 Na_2HPO_4 often produces poor inoculum, whereas only moderate crushing of the same tissue produces good inoculum; and that 2) addition of healthy juice to good inoculum prepared with M/10 Na_2HPO_4 often inhibits the virus, or at any rate reduces the effectiveness of the inoculum. Fulton has reported that inoculum from local lesions on tobacco leaves, presumably prepared by thorough grinding in a mortar, deteriorates rapidly with aging, and that the addition of M/100 Na_2SO_3 prevents or retards the aging.

In the present study, inoculum prepared by only moderate or slight crushing of chronically affected leaves of *N. rustica* did not become inactivated markedly by aging in the manner that Fulton reports, probably because the leaves were not crushed thoroughly enough to release the precursor material which upon oxidation inhibits the virus. Perhaps at the times when inoculum from *N. rustica* tissue was not very active, excessive crushing or some other unknown condition released more of the inhibitor precursor. The addition of Na_2SO_3 during preparation of inoculum facilitates the preparation of inoculum of high titre.

SUMMARY

The streak virus has been relatively difficult to transmit mechanically, especially to resistant species of *Nicotiana*. Usually inoculum with high virus titre, as measured by the local lesions produced on rubbed tobacco leaves, resulted when relatively young leaves of chronically diseased plants of *N. rustica* were moderately crushed with a glass spatula with approximately an equal volume of M/10 Na_2HPO_4. However, inoculum of high titre did not always result. The effects of age of prepared inoculum, ages of leaves used for inoculum, vigor of plants used for inoculum, age of leaves rubbed, and amount of rubbing were tested and eliminated as possible causes for variability. However, very thorough grinding of the inoculum often reduced its virus activity. Also, the addition of healthy juice to inoculum usually inhibited the virus. Na_2SO_3 added to inoculum prepared from young leaves of chronically diseased *N. rustica* eliminated the deleterious effect of thorough crushing. Inoculum of high titre was produced consistently by crushing young leaves of *N. rustica* with M/10 Na_2HPO_4 containing M/100 Na_2SO_3, or with M/100 Na_2SO_3.

KENTUCKY AGRICULTURAL EXPERIMENT STATION
LEXINGTON, KENTUCKY

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THE LIFE HISTORY AND MORPHOLOGY OF THE SUGAR-BEET NEMATODE, HETERODERA SCHACHTII SCHMIDT[1]

D. J. Raski[2]

(Accepted for publication August 4, 1949)

INTRODUCTION

The sugar-beet nematode, *Heterodera schachtii* Schmidt, has been a serious problem in the production of sugar beets for almost 100 years. It is present in all the major sugar-beet-producing areas in the world. The annual losses due to this pest range from partial decreases in production to total failure of a crop. In addition, the enforced rotation program due to the presence of heavy infestations of this nematode is also an economic loss to growers in some areas.

Since its discovery in 1859, many contributions have been made concerning this pest. Yet there is no close agreement as to the stages in the life cycle of the nematode or in the length of time required for the development of each stage. Most illustrations of the various stages in the life history of the nematode have been reproductions of work published in 1888. Many of these illustrations are somewhat incomplete and inaccurate.

The present work is an attempt to resolve the statements found in the literature and to add to the knowledge concerning the life history and morphology of the sugar-beet nematode.

HISTORICAL REVIEW

The first report of the sugar-beet nematode was made by Schacht (15, 16) in 1859. Although the parasite was identified as a new species, Schacht did not describe or name it. In 1871 Schmidt (17) erected the genus *Heterodera* and named the species *H. schachtii* in honor of Schacht.

The first thorough treatment of the development and structure of the sugar-beet nematode was presented by Strubell (19). His drawings are still the most complete available and have been used by many subsequent authors as illustrations of the various stages of the nematode. Strubell concluded that there were two stages in the development of the female and three in the male. In the text of his article Strubell actually describes three stages in the development of the female and five stages in the male. A molt inside the egg and another in the development of the male were described but not considered as separate stages.

Chatin's report (3), including illustrations, is almost identical to that of Strubell, but is not so complete or so accurate. He described the molt

[1] Presented as a thesis to the graduate division of the University of California, June 1948, in partial satisfaction of the requirements for the degree of Doctor of Philosophy.

[2] Instructor and Junior Nematologist in the Experiment Station.

The writer is indebted to Dr. M. W. Allen, under whose direction this research was undertaken, for his stimulating and valuable advice and for his constant encouragement throughout the investigation.

within the egg but did not consider it as a separate stage in the development. Marcinowski (13) refers to Strubell's description of the molt within the egg, but in subsequent literature there is no further reference to this molt. According to Nagakura (14), a similar molt occurs within the egg of the root-knot nematode, *Heterodera marioni* (Cornu). This was later verified by Christie and Cobb (5). In the closely related species, *H. rostochiensis* Wollenweber, Chitwood and Buhrer (4) report they were unable to find a molt inside the egg.

Cobb (6) published an illustrated comparison of the free-living infective larvae and adult males of *Heterodera marioni* and *H. schachtii*. Cobb suggested that the difference between the free-living larvae of *H. schachtii* taken from sugar-beet fields in Colorado and the larvae taken from sugar-beet fields in California was due to a variation in the time of molting. Unfortunately, some of the nematodes in the Colorado collection were of another species which probably belonged to the genus *Nacobbus*, according to Thorne and Allen (23). As a result, the illustrations showing the blunt-tailed larvae and the male tail are not typical of *H. schachtii*.

Thorne (22) described and illustrated a collection of *Heterodera schachtii* from shadscale, *Atriplex confertifolia* (Torr. and Frem.) S. Wats., in Utah. Thorne has since indicated (unpublished correspondence, 1948) that this identification is doubtful because of the form of the male tail and the markings on the wall of the brown cyst.

Goodey (11) describes two molts and three stages in the development of the male and female nematodes of this species. Filipjev and Stekhoven (8)

LEGEND FOR FIGURES 1 THROUGH 4

amph—amphid
an—anus
bdy cav—body cavity
c—tail
chlrbn—cheilorhabdion
cr st—cross striation
ctl—cuticle
dev sp—developing spicule
dsl gl—dorsal gland
dsl gl ap—dorsal gland aperture
dsl gl ncl—dorsal gland nucleus
em ov—embryonated egg
ex p—excretory pore
gel mtr—gelatinous matrix
gen prm—genital primordium
ger zn—germinating zone
gr zn—growth zone
gub—gubernaculum
int—intestine
int inc—intestinal inclusion
lat fld—lateral field
lp—lip or lip region
lu oe—lumen of esophagus
med blb—median bulb
mlt ctl—molted cuticle
mlt prbn—molted prorhabdion
ms-mtrbn—meso-metarhabdion
n—neck
ncl—nucleus
nrvr—nerve ring
oe gl—esophageal gland
oe crp—corpus
on—spear
or ap—oral aperture
ov—egg
ovry—ovary
ph—phasmid
post oe—posterior part of esophagus
ppl—papilla
prbn—prorhabdion
ret—rectum
sp—spicule
subm gl—submedian gland
tlrbn—telorhabdion
tst—testis
vag—vagina
vlv—valve
vul—vulva

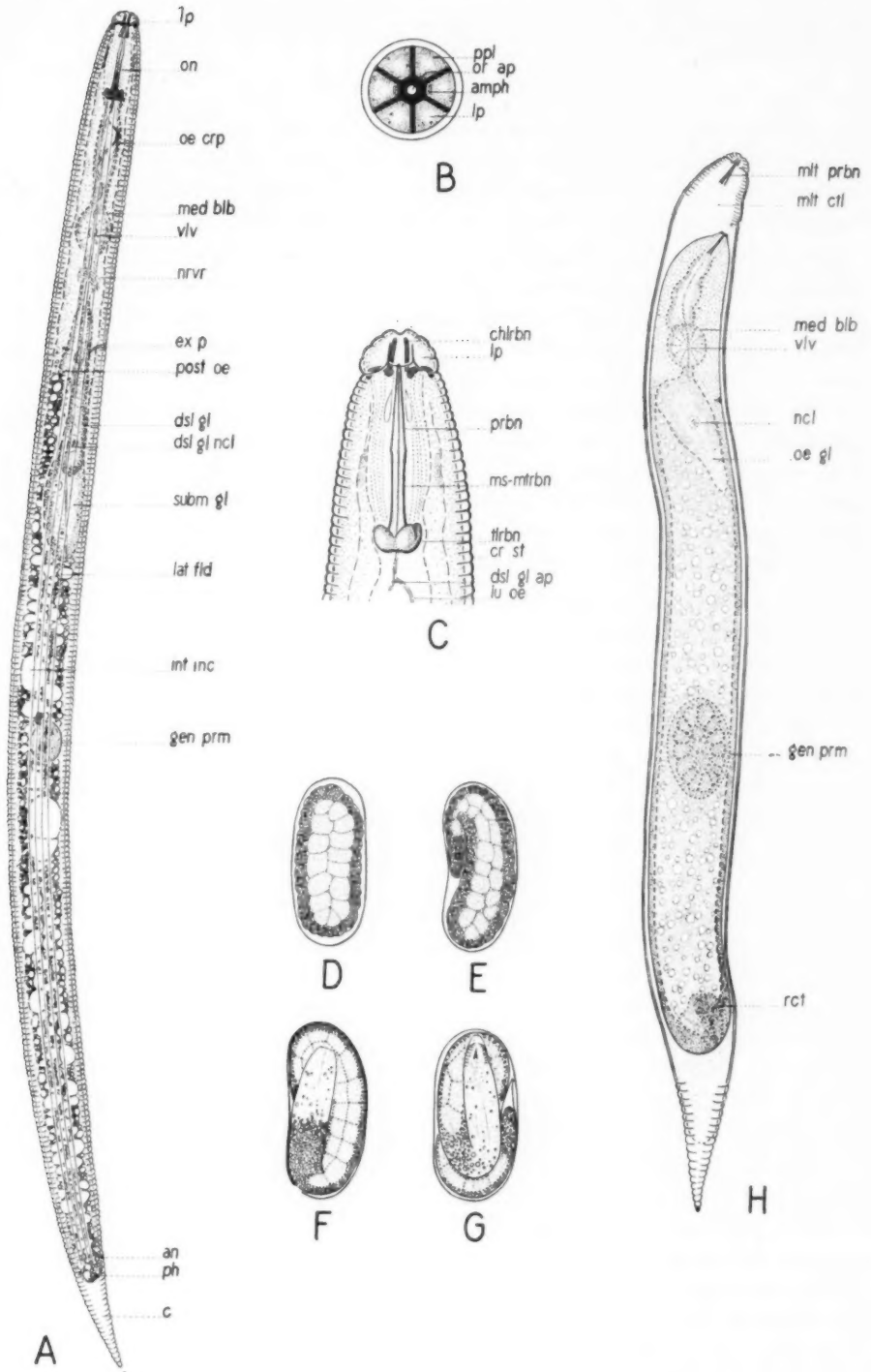

FIG. 1. A. Second-stage larva (330×). B. Face view of second-stage larva (1300×). C. Head view of second-stage larva (1000×). D. Embryo just prior to elongation (180×). E. Embryo elongating with one flexure (180×). F. Fully developed first-stage larva (180×). G. First-stage larva molting (180×). H. Second-stage larva molting (330×).

recognize three molts and four stages but make no reference to a molt within the egg.

MATERIALS AND METHODS

Embryonic development. Larvae in the process of molting in the egg were secured by selecting females which had grown to maturity and were just beginning to turn brown. These females, which contained eggs in all stages of development, were placed in a drop of water on a slide and the eggs teased out. The fragments of the female were then removed and a coverglass dropped in place over the eggs. Gentle pressure applied to the coverglass with a needle ruptured many of the eggs without damaging the larvae. In this way a number of larvae which demonstrated the molt could usually be found on each slide.

Postembryonic development. The development of the nematode inside the roots was followed by mixing infested soil half and half with sand and transplanting into the mixture 100 sugar-beet seedlings that had been germinated in sterile soil. These were allowed to grow for 24 hr., then all the seedlings were washed free of soil and transplanted to sterile soil. On each succeeding day for 5 weeks, two seedlings were washed free of soil and the roots stained with acid fuchsin in lactophenol according to the procedure recommended by Goodey (12). The daily development of the larvae was then observed by removing the larvae from the roots and mounting them in glycerine for microscopic examination.

Throughout this period of development, continuous soil temperature records were obtained. The recordings showed a daily maximum temperature varying from 65° to 80° F., with an average of 74.4° F. The average daily temperature taken from hourly recordings for each 24-hr. period was found to vary between 62° and 70° F. The total hourly average was 66.8° F.

DESCRIPTION OF LIFE STAGES

Embryonic development. Prior to elongation of the embryo the egg is composed of a large number of cells in which two layers can be distinguished (Fig. 1, D). According to Strubell (19), the cells of the inner, double row are the mesoderm and the outer cells are the ectoderm.

As the embryo increases in length it becomes more slender and the growth is accompanied by active movement. The first reflex is shown in figure 1, E. The progressive increase in length results in two and three flexures of the embryo. There is very little differentiation in the head region of the fully developed first-stage larva (Fig. 1, F).

The first molt (Fig. 1, G) occurs soon after the embryo achieves full growth in length. The cast skin separates and cross annulations and the spear begin to form at the anterior end of the second-stage larva. The prorhabdion is slightly curved when first forming in some specimens. This curvature is not apparent in most specimens and the typical V-shape of this part of the spear is developed. There is a great deal of variation in the

development of the lip region at this time. In some specimens it is already definitely set off, while many larvae show intermediate steps in the formation of the lip region.

The second-stage larvae complete their development before hatching from the egg. The larvae may hatch from eggs that have been deposited in the gelatinous matrix secreted by the female, or from eggs retained inside the body of the female when it changes to a brown cyst. In the latter case the larvae escape through the vulva of the dead female. The larvae then begin their free-living stage and search for a suitable host plant root.

Second-stage larva. The second-stage larva (Fig. 1, A) varies in length from 0.435 to 0.492 mm. and in width from 0.021 to 0.022 mm. The annular striations of the cuticle are moderately coarse, those opposite the spear being about 1.4 μ wide, and those at the middle of the body about 1.7 μ wide. The lateral field is marked by four incisures which start as three lines at a point slightly posterior to the base of the spear and soon increase to four lines extending to a point slightly posterior to the anus. The lip region is definitely set off by constriction and bears three cross striations. The lip region, as seen in face view (Fig. 1, B), is divided into six sectors. The amphid apertures are minute, slitlike openings on the lateral lips close to the oral opening. Each subdorsal and subventral lip has a single obscure papilla located medially on the outer margin. The cephalic framework or cheilorhabdion is heavily sclerotized. The robust spear is about 25 μ long (Fig. 1, C). The genital primordium is located slightly posterior to the middle of the body, but is obscure in newly hatched larvae. The primordium consists of two nuclei in a protoplasmic mass that is not visibly separated into two cells. The obscure phasmids terminate laterally as small circular structures slightly posterior to the anus. The body tapers gradually from the anus to form a rounded tail.

When the infective larvae penetrate the roots of a suitable plant they migrate a short distance in the cortex. After reaching their final position the larvae are usually parallel to the longitudinal axis of the root with the tail toward the root tip. The larvae remain in the cortex but assume a position with the head near the vascular system. This stage completes its development in the greenhouse about seven days after penetrating sugar-beet roots.

Second molt. Evidence of the second molt (Fig. 1, H) was found 6 days after penetration of the roots. One larva of the 12 examined was in the process of molting. Eighteen larvae examined on the 7th day were all in the process of molting.

The total length including the cast skin is approximately 0.420 mm., slightly shorter than the free-living, second-stage larva. The third-stage larva forming within the cast skin of the second-stage larva is approximately 0.346 mm. in length and 0.030 mm. in width. The genital primordium is approximately 37 μ in length and, in some larvae, occupies almost the entire

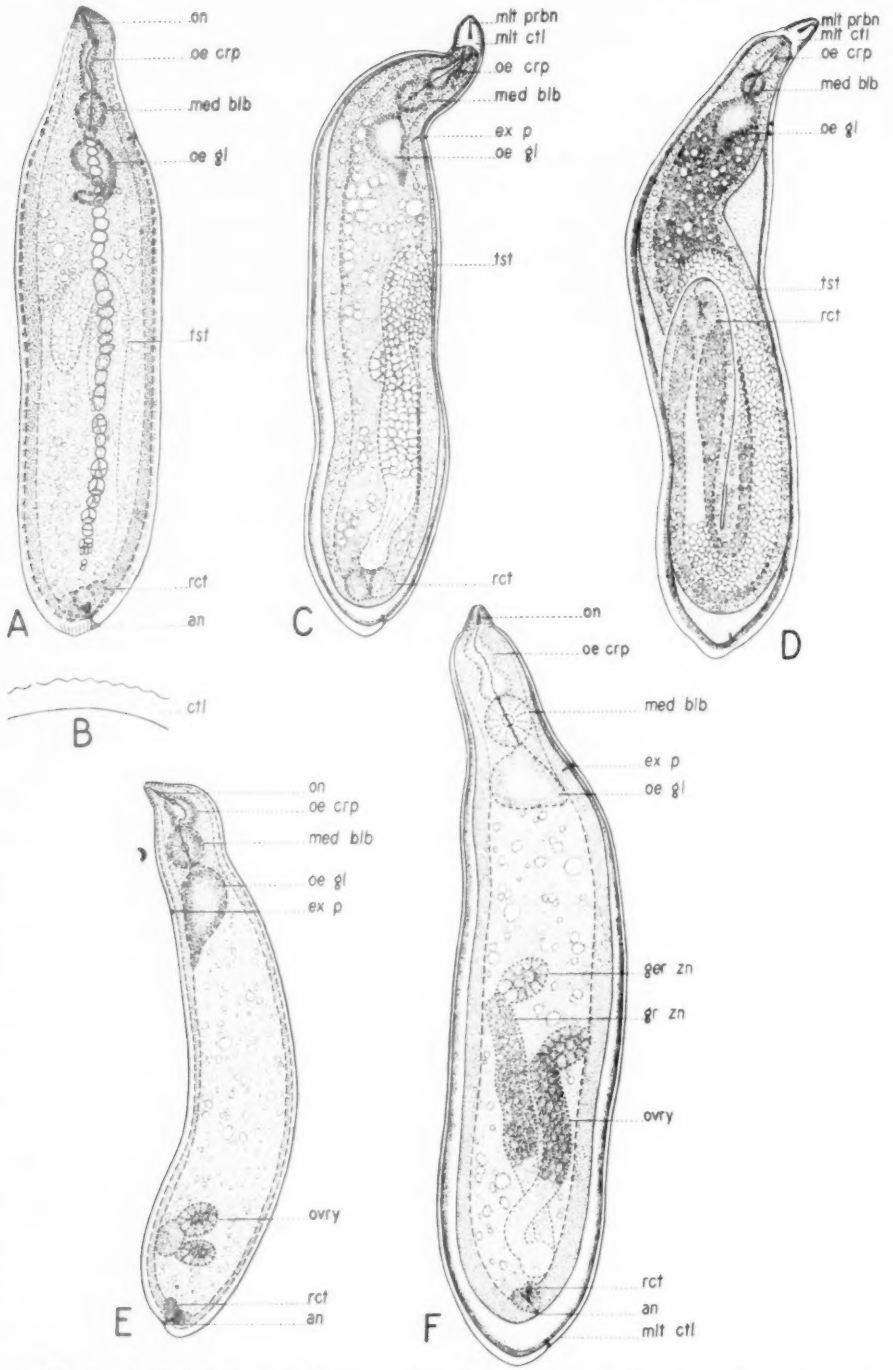

FIG. 2. A. Third-stage male larva (250×). B. Cross section of cuticle of third-stage male larva (1335×). C. Third-stage male larva molting (250×). D. Early fourth-stage male larva (250×). E. Third-stage female larva (250×). F. Third-stage female larva molting (250×).

width of the body cavity. The rectum is well developed. According to Chitwood and Buhrer (4), the rectum in this stage is consistently larger in the male of the golden nematode of potato, *Heterodera rostochiensis*, and can be used to differentiate sexes. No such correlation could be established in *H. schachtii*.

Twelve larvae were examined on the 8th day, seven of which were still in the process of molting. Five larvae had completed the molt and were increasing in size. All the larvae completed the second molt by the 9th day. The sexes can be distinguished by the time the second molt is completed and the development of each sex followed separately. The females develop two ovaries, whereas the formation of the single male testis is quite different (Figs. 2A, 2E). According to Filipjev and Stekhoven (8), the third stage larva does not possess characters that enable the recognition of sexes, but there are no illustrations that can be used to compare the morphology of the stage they describe and the development as found here.

Third-stage male larva. The fully developed third-stage male larva (Fig. 2, A) varies from 0.324 to 0.377 mm. in length and 0.057 to 0.085 mm. in width. It is slightly more robust than the third-stage female larva but is still rather slender in its general appearance. The head region has 7 to 8 very narrow cross annulations and tapers almost to a point. The spear is 16–19 μ long and is much less robust than the spear of the second-stage larva. The basal swellings are not distinct knobs but appear to be simple enlargements of the base of the spear. The median bulb is slightly larger than the median bulb in the second-stage larva, but not so large as the bulb in the larva of the third-stage female. Another important development at this stage is the growth of the genital primordium. The first change occurs in the elongation of the anterior end which becomes reflexed. At the same time a posteriad elongation of the posterior end has started. This elongation reaches a point slightly anterior and ventral to the rectum. Internally there are two rows of cells that were found only in the third-stage male larvae. These cells are located laterally beneath the cuticle and extend from the level of the excretory pore almost to the rectum. The nature of these cells is not known but it is possible that they are in some way related to the lateral chords in the developing male. The cuticle has no distinct markings except for the narrow annulations on the anterior end as already described. A cross section (Fig. 2, B) made approximately midway in the body of the larva showed irregular elevations and depressions in the surface of the cuticle. The third-stage male larva completes its development 11 days after penetration of the roots.

Third molt of the male larva. Specimens which showed the first signs of the third molt (Fig. 2, C) were found on the 10th day of development, and by the 11th day nine out of eleven specimens were in the process of molting. The male larva casts the third larval skin by shrinking within it, and occupies the entire length of the old larval skin. The posterior tip of

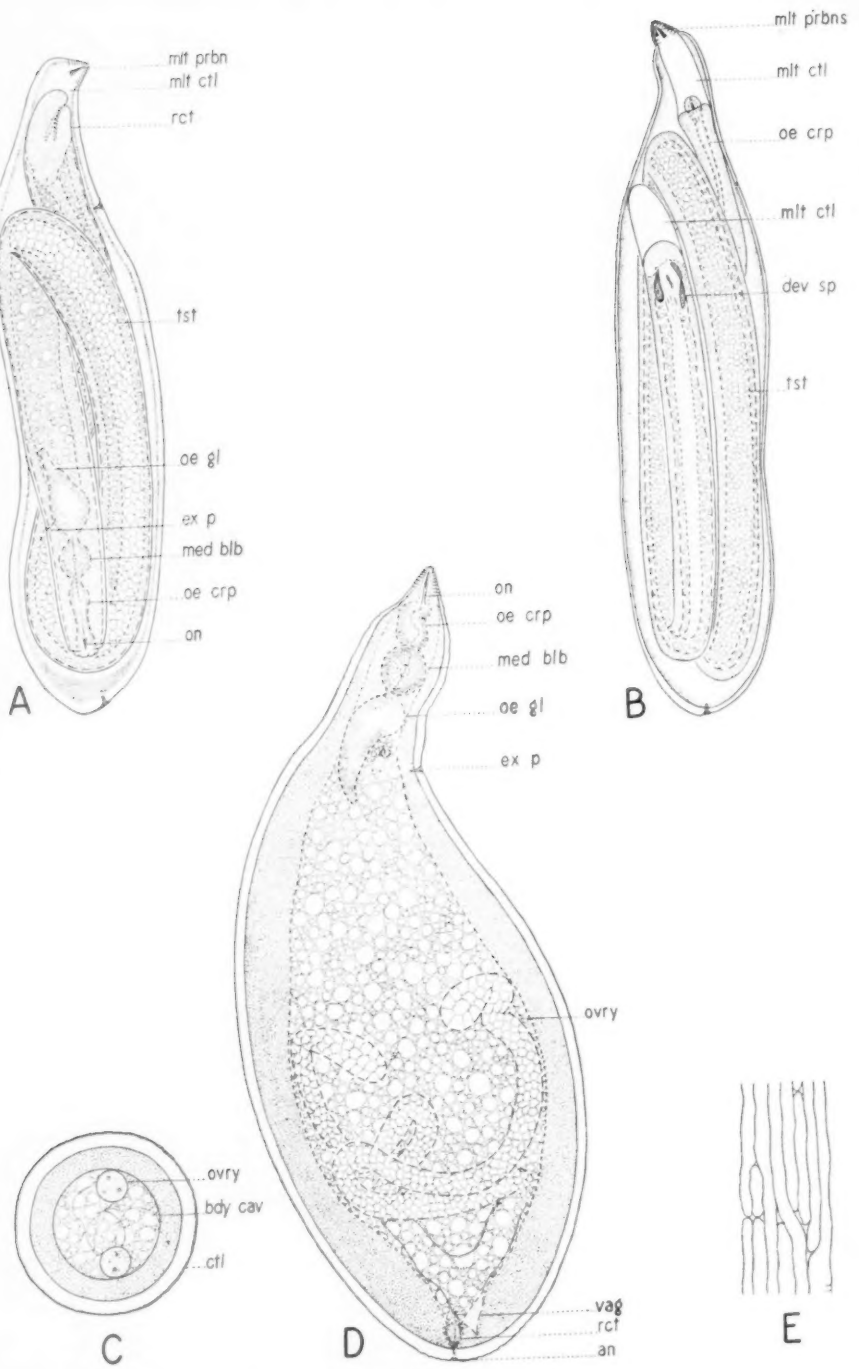

FIG. 3. A. Advanced fourth-stage male larva (250×). B. Fourth-stage male larva molting (250×). C. Cross section near posterior end of fourth-stage female larva (250×). D. Fourth-stage female larva (250×). E. Markings on cuticle of fourth-stage female larva (500×).

the developing testis is located immediately anterior to the rectum. The rectum appears to be subterminal in a median position internally but the anus is not visible. There are no markings on the new cuticle.

Fourth-stage male larva. Fourteen out of 17 specimens examined on the 12th day of development had cast the third larval skin and were in various stages of growth by elongation. This growth begins soon after the third skin is cast and is accompanied by the formation of several coils within the cast skin. The first suggestions of a lip region are evident by the time the larva has formed the first reflex (Fig. 2, D). The posterior end of the developing testis has made intimate contact with the rectal region and appears to be continuous with it. The rectum is still subterminal internally but the anus cannot be distinguished.

Three coils are formed within the third-stage cuticle by the fourth-stage larva by the 12th and 13th days. This development represents nearly the full growth of this stage (Fig. 3, A). The lip region is more evident by this time and is represented by several small lobes. The rectal region has gained access to the exterior through the subterminal anal opening on the ventral side. The full development of the fourth stage is completed after about 14 days of growth in the roots.

Fourth molt of the male larva. Evidence of the fourth molt of the male nematode was found as early as the 12th day. The larvae examined on the 14th day were all in the process of the fourth molt (Fig. 3, B). Three reflexes are formed and the larva extends the length of the interior of the third larval skin four times. The developing spicules and gubernaculum are visible for the first time but the rectum is not clearly defined.

The larvae complete the fourth molt by the 15th day and the adult males are coiled in the third larval skin. The lip region is distinct and the spear has started to form. The prorhabdion is fully developed but the rest of the spear is not completed until the 17th day. The spicules are well developed and cross striations are in the process of formation in the cuticle.

Adult male. The adult male is slender and wormlike. It is 1.119–1.438 mm. in length and 0.028–0.042 mm. in width. The cuticle is marked with cross striations over the length of the body (Fig. 4, A). The lateral field is marked by four incisures which start at a point slightly posterior to the base of the spear. The incisures begin as three lines but soon increase to four and extend the length of the body. Posteriorly the incisures separate slightly as they extend around the blunt tail, and the two lateral fields meet at a mid-line on the tail (Fig. 4, B). The lip region is clearly set off by constriction and has three cross striations. The lip region in face view (Fig. 4, C) closely resembles the second-stage larva except for the lobed appearance of the sectors, which appear as a smooth circle in the larva (Fig. 1, B). The cephalic framework is heavily sclerotized. The robust, hollow spear is 29 μ long. The dorsal esophageal gland and its duct can be distinguished in some specimens from the subventral glands. The testis

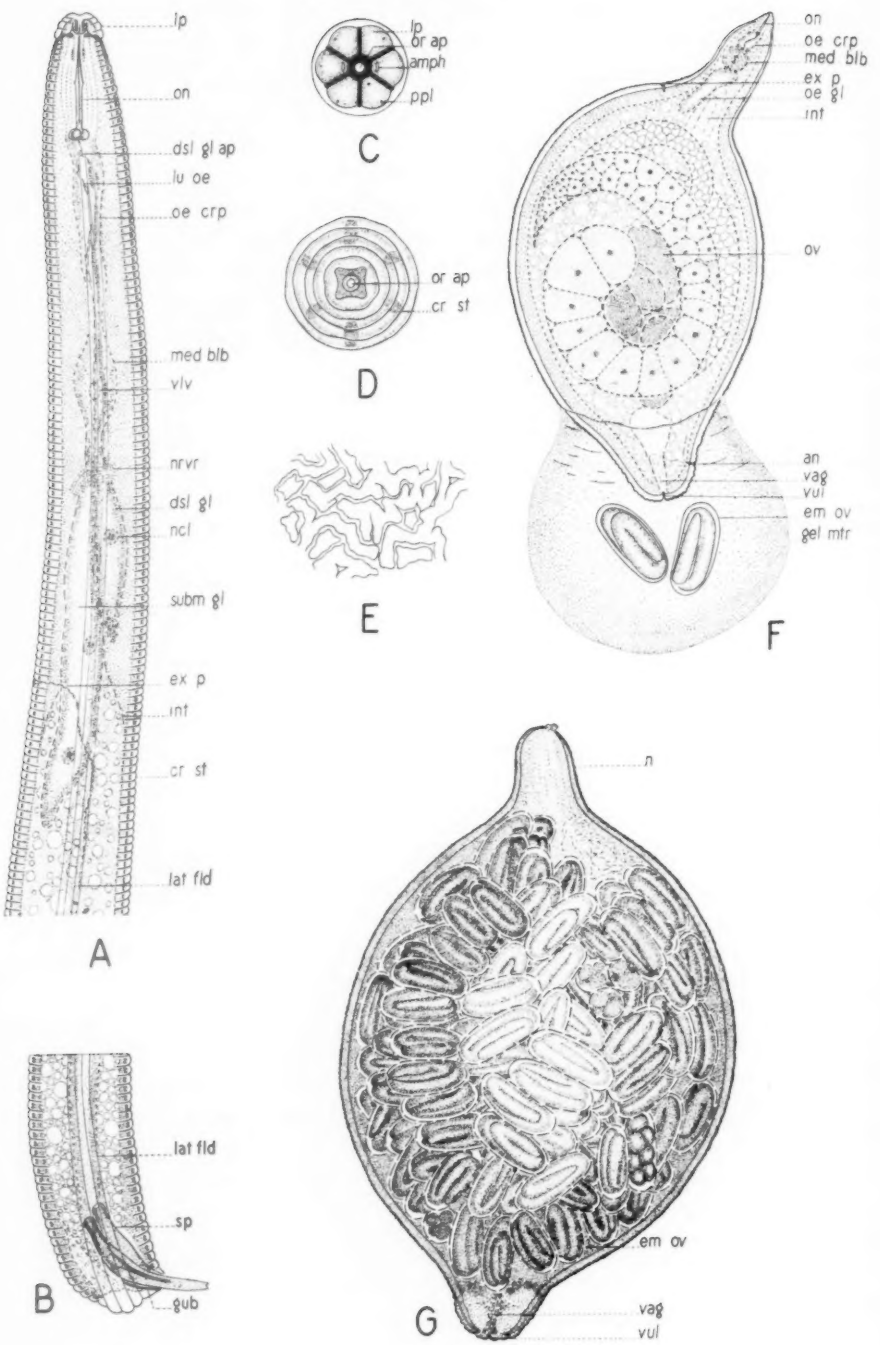

FIG. 4. A. Neck region of male (500×). B. Posterior end of male (500×). C. Face view of male (1300×). D. Face view of female (1300×). E. Markings on cuticle of female (650×). F. Female (100×). G. Brown cyst (100×).

is about 0.953 mm. long. There is no structure that corresponds to the rectum in the larval stages. The slightly arcuate spicules are 34–38 μ long and bear a single notch at the tip. The gubernaculum is simple, 10–11 μ long.

The last collection of males was made 19 days after penetration of the roots. The adult males presumably had all emerged from the third larval skin and had left the roots. The activities of the male after emergence from the roots are apparently of short duration. Most authors agree with Strubell who states the males live only a few days and soon disappear from the roots and soil. It is also generally agreed that fertilization occurs as a normal procedure but there is no evidence that it is essential in the reproduction of this species. Populations of *Heterodera marioni* have been maintained from single larvae for several generations by Tyler (25). There is no published work on attempts to do the same with *H. schachtii*.

Third-stage female larva. The larva of this stage (Fig. 2, E) varies from 0.320 to 0.366 mm. in length and 0.044 to 0.070 mm. in width. It is still somewhat slender in appearance compared with its later development but much stouter than the second-stage larva. The third-stage female larva possesses the characteristic head structure described in the third-stage male larva. This development is retained throughout the adult stage. The spear is 22 μ long and is less robust than the spear of the second-stage larva. The median bulb of the female is consistently larger than the median bulb of the developing male in this and all subsequent stages. The ovaries begin to develop by the growth of two oval enlargements arising from the genital primordium. These are the germinating zones of the ovaries. There are no cuticular markings except for the annulations of the head region. The third-stage female larva reaches full growth 11–12 days after penetration of the roots.

Third molt of female larva. Five of the seven larvae examined on the 11th day were in the process of the third molt and nine out of ten had completed the third molt on the 12th day. The major change that occurs by the time of the third molt is the rapid increase in the length of the ovaries (Fig. 2, F). The structure that corresponds to the genital primordium from which the two ovaries develop is in a ventral position slightly anterior to the rectum. In some cases it is adjacent to the rectum. The cuticle of the fourth-stage larva develops irregular longitudinal markings during the third molt. These markings do not extend onto the head region, which retains the cross annulations.

Fourth-stage female larva. The female larva loses its somewhat slender appearance in the fourth stage and develops the typical flask shape (Fig. 3, D). The fourth stage varies from 0.396 to 0.419 mm. in length and 0.115 to 0.176 mm. in width. The internal structure of the larva presents what appears to be a body cavity surrounded by a broad layer of undifferentiated granular tissue. The exact nature of this tissue is not certain. Strubell

illustrates the intestine of the adult female occupying almost the entire space within the cuticle of the nematode. He shows the coiled ovaries growing between the cuticle and the intestine pressing it inward to accommodate the ovaries. By a cross section made near the posterior end of a fourth-stage larva (Fig. 3, C), it was established that the ovaries develop throughout the body cavity, which corresponds to the intestine illustrated by Strubell. The same condition is found in the adult female. The vagina is ventral to the anus and rectum and extends almost to the cuticle. The rectum and anus are actually located dorsally in this stage. The cuticle is marked by longitudinal lines which give the appearance of irregular narrow bands running the length of the body (Fig. 3, E). These bands become somewhat distorted around the posterior end of the body. Development of the fourth-stage female larva is completed in 15 to 16 days after penetration of the roots.

Fourth molt of female larva. The first evidence of the fourth molt was observed on the 15th day of development. On the 16th day at least one female was fully developed into an adult nematode. Six adult females were found on the 17th day with four others in the process of molting. During this molt the reproductive system gains access to the exterior by the formation of the terminal vulva.

Adult female. The adult female soon grows to the typical lemon shape (Fig. 4, F) and varies from 0.626 to 0.890 mm. in length and 0.361 to 0.494 mm. in width. The head region remains unchanged. In face view (Fig. 4, D) there appears to be a circumoral elevation and then a square area which may or may not be all that remains of the lip region. The annulations appear to surround this area as concentric circles of increasing diameter. The circles appear to be somewhat irregular but it is very difficult to follow their exact outlines. Six radially arranged structures can be discerned by focusing through the face view. These are internal and may possibly represent the remnants of organs that once innervated the six lips but were reduced and changed when the lip region was modified into the present form. The spear is very slender and about 27μ long. The median bulb of the adult female is much larger than in any previous stage and varies from 28 to 30 μ in length and 27 to 30 μ in width. The ovaries fill almost the entire body cavity and it is no longer possible to follow the convolutions throughout their entire length. The rectum is not well developed in the adult female. The vagina is enlarged anteriorly, then tapers slightly as it extends to the vulva. Various sclerotized structures extend from the middle of the vagina to the walls of the body. These structures persist in the brown cyst after the death of the female. The granular layer of tissue surrounding the body cavity in the fourth stage is also present in the adult. The cuticle varies from 9 to 12 μ in thickness. In the adult the arrangement of the markings on the cuticle gives the appearance of wavy, irregularly disposed bands (Fig. 4, E). There appears to be a cast skin

clinging to the cuticle of most specimens of the adult female. This was thought to be a subcrystalline layer by Schmidt (17). Strubell (19) later identified it as the cast skin of the fourth-stage larva. Thorne (unpublished correspondence, 1948) believes this layer to be a separate production of the adult nematode, but the exact nature of the layer and its function have not been determined.

A gelatinous matrix, which is usually covered with dirt and debris, surrounds the posterior end of the adult females. Most of the females examined here had deposited eggs in this matrix. The number of eggs in the matrices varied from a few to 131. The numbers found in the upper part of the range were made up of counts from both empty eggs and embryonated eggs. The mass with 131 eggs, for instance, contained 103 empty shells and 28 embryonated eggs. Almost all the unhatched eggs in the matrices had developed into fully coiled second-stage larvae. From the number of empty egg shells it is evident that the larvae do not remain in the matrix long before emerging to search for suitable host plant roots.

The changes in the adult female after the 18th day are an increase in size and the further development of the ova to fully formed eggs. The ova near the distal ends of the ovaries enlarge considerably by the 19th day, and by the 21st day two eggs of nearly full size were found. Five eggs were observed on the 24th day, one of which was in the two-celled stage. The number of eggs varied from 4 to 23 in the individuals observed on the 25th day. There was a gradual increase in the number of eggs developed within the body until the 30th day, by which time the eggs completely fill the body cavity of the females. Eggs containing coiled larvae appear in adult females by the 31st day after root penetration.

Brown cyst. The first brown cyst (Fig. 4, G) was found on the 36th day of development on the roots. Three cysts were collected on the 39th day. The eggs in these cysts contained fully developed and coiled larvae. One of the cysts held two larvae that had hatched and were still within the cyst. All three cysts were partly empty. This indicated that part of the eggs had probably hatched and the larvae had emerged from the cyst body.

The formation of the brown cyst as reported by Chatin (1, 2) occurs by the exudation of a brown liquid produced by glands of the epidermis. He believed that the viscous and refractive globules are emitted through ruptures in the epidermis, not through pores, and that this material congeals over the surface of the body of the female, uniting the fragments of the body epidermis into the brown cyst. Fuchs (10) did not agree with these conclusions. He found exudations on portions of the epidermis not ruptured and concluded the liquid is produced through pores. Both Fuchs and Chatin believed that only certain individuals turn into brown cysts. The others are dispersed into the soil, where they disintegrate and release their larvae and eggs. Sengbusch (18), however, was able to show that all the females that are exposed to the atmosphere turn to brown cysts re-

gardless of the time of year. Ellenby (7) demonstrated that the process of hardening in the brown cysts of *Heterodera schachtii* and *H. rostochiensis* is due to the activity of polyphenol oxidase. The process is said to be similar to the formation of insect cuticle. This transformation of the cuticle into the brown cyst results in a sac that persists in the soil many years after all the eggs have hatched. This characteristic makes possible the use of the cyst flotation method (21) in determining whether or not a field is infested with sugar-beet nematode. The surface of the brown cyst shows markings somewhat similar to those found on the adult female. The lines, which are also irregularly arranged, are possibly slightly more angular on the brown cyst than on the cuticle of the white female adult. Franklin (9) reports observations of minute pits on the surface of brown cysts of several *Heterodera* species. According to Franklin, the pits are irregularly arranged on *H. schachtii* and beneath the pits are the normal markings. A thorough examination of brown cysts was made in these studies, but the presence of the minute pits referred to by Franklin could not be verified.

The size of the brown cyst is determined by the extent of the growth of the female before death occurs. The cysts that were measured all fell within the range of measurements taken on the white adults. Soon after the formation of the brown cyst the remnants of the digestive system, including the esophagus, median bulb, esophageal glands, and intestine, disappear by collapsing and disintegrating. Finally the brown cyst becomes only a shell enclosing eggs. The number of eggs retained in the brown cysts was found to be as high as 605.

Number of generations per year. One aspect of the life history of the sugar-beet nematode which is reported upon variously in the literature is the number of generations produced each year. Strubell (19) estimated that six to seven generations could be produced in one year. Thorne (21) states there are several generations each season in the United States. Triffitt (24) concludes that there is one and at most a partial second generation produced under conditions found in England. There are two factors that should be considered. The first is the eggs deposited in the gelatinous matrix, and the second, the eggs held in the brown cysts. The fact that 100 or more eggs may be deposited in the matrix, all of which are capable of immediately producing viable larvae, cannot be disregarded from the standpoint of producing another generation. With the exception of Thorne (22, 23), most authors have considered the problem of generations as primarily concerned with the eggs held in the brown cysts.

The activities of the larvae held in the bodies of adult female nematodes were observed in the following manner. White females were removed from the roots of sugar-beet plants and placed in water. The nematodes were carefully collected and handled to avoid rupturing the females. The matrices were removed to eliminate any eggs or larvae on the outside of the body. Twenty-five adults were placed in the soil in each of two 4-inch

pots. Three days later, sugar-beet seeds were planted in one pot and allowed to grow for 3 weeks. At the end of that time the seedlings were washed free and stained. All were penetrated by larvae of the sugar-beet nematode. One month later this procedure was repeated with the other pot of soil and the same results were obtained. This indicates that the larvae are able to emerge from the females and penetrate roots soon after the females are removed from the roots of the plants. The soil was kept moist by frequent waterings during the experiment.

In another series of observations, sugar-beet seedlings were grown for 3 weeks in soil infested with sugar-beet nematode, then washed free of soil and transplanted to sterile soil. One month later the roots of five seedlings were examined. All the females on four of the seedlings were mature white females. The original roots of the fifth plant seemed to have suffered from transplanting and were dead or dying. The females on this seedling had turned to brown cysts. The eggs in the cysts were in all stages of segmentation; some held coiled embryos but none held completely formed larvae. Two months after transplanting the plants to sterile soil, brown cysts were found on three of the five seedlings examined. Eight white females and 28 brown cysts were counted. Almost all of the larvae in 21 of the 28 brown cysts had hatched from the eggs and emerged from the cysts. Two of the white females examined were in the early process of forming eggs, the other six were full of eggs, of which 50–80 per cent contained fully developed second-stage larvae. There were 23, 371, and 457 larvae, respectively, found in the roots of each of the three seedlings with brown cysts. The larvae were so numerous in some of the rootlets that it was not possible to make accurate counts, and the figures shown represent only the minimum number present. These larvae were almost all found in the rootlets in the near vicinity of the brown cysts.

From these results it appears that in the presence of abundant soil moisture, favorable temperatures, and a suitable source of food supply, all the larvae may hatch from the eggs contained in brown cysts and emerge to penetrate the roots of suitable plants. It is probable that cysts formed during the latter part of the growing season are subjected to conditions of unfavorable temperature and/or moisture and the absence of food material. The nematodes then overwinter as fully developed second-stage larvae in the eggs contained in cysts. Most, if not all, of the larvae hatch from the eggs and emerge when conditions are again favorable. Some larvae may emerge in the absence of a suitable host plant when soil, temperature, and moisture conditions are favorable, but apparently not all hatch the same year. Thorne (20) and Fuchs (10) present data which show a gradual decrease in the number of viable eggs held in brown cysts over a period of years. The information was obtained by examining fields infested with nematodes but free of suitable hosts for sugar-beet nematode for periods varying from 1 to 12 years. Their results indicate that a few larvae hatch each year under

the conditions described, until practically all have emerged by the end of the sixth year.

SUMMARY

The life-cycle of the sugar-beet nematode includes five stages and four molts. The first stage is completed inside the egg and the first molt occurs soon after the embryo becomes elongated and wormlike. The second-stage larva is completely formed before it hatches from the egg. The free-living stage is characterized by its typical wormlike form, coarsely annulated cuticle, distinct lip region, and robust spear.

After penetrating suitable roots the larvae undergo three molts before reaching the adult stage. The following periods of development occurred in the greenhouse at an average soil temperature of 66.8° F. Seven days after entering the roots of sugar-beet seedlings the second molt occurred. Sexual differentiation of the third-stage larvae can be made by the median bulb, which is larger in the female, and the double ovaries developing in the female as distinguished from the single testis in the male.

In general the third stage is characterized by a more robust but still elongated shape, small spear, and short, finely annulated head region. The third molt takes place 4 days after the second molt, following which the development of the male and female nematodes differs considerably.

The male larva molts for the third time within the third larval skin and then completes its development inside this cast skin. The larva grows in length to form several reflexes and has few distinct characters. The fourth molt occurs approximately 3–4 days after the third molt.

The slender adult male is characterized by its well developed lips, knobbed spear, distinct cross striations, four incisures in the lateral field, and blunt tail. The well-developed spicules bear a single notch at the tip. Development of the adult male is completed in about 3–4 days after the fourth molt and the nematodes emerge from the third larval skin to leave the roots. The development of the adult male thus requires 18–19 days and most of them emerge by the 20th day.

The female larva develops after the third molt by rapidly growing in size but retains many of the characters of the third stage. In general the larva is flask-shaped with a rounded posterior end and the cuticle has faint longitudinal markings. The reproductive system has no opening to the exterior and the anal opening is dorsally located.

The fourth molt takes place 3–4 days after the third molt. The profound change which takes place in this molt produces the typical lemon-shaped adult female. The reproductive system gains access to the exterior through the terminal vulva. The anal opening is dorsally located. The head region is short and finely annulated and has no lips. The spear is slender, the median bulb large, and the ovaries large and highly convoluted. The cuticle is marked with wavy bands irregularly disposed. At the posterior end a gelatinous matrix is produced in which eggs may be deposited. The devel-

opment to adult female is completed in 16–17 days after penetration of sugar-beet roots, but the first eggs do not develop until the 23rd or 24th day. Some females are completely filled with eggs by the 30th day. There are coiled larvae inside the eggs by the 31st day. As many as 131 eggs were found deposited in the gelatinous matrix.

The first brown cysts were found on the 36th day and by the 39th day the cysts observed held eggs all of which contained coiled larvae. There were as many as 605 eggs observed in the brown cysts. Whenever conditions are favorable, larvae may hatch from eggs in the brown cysts soon after the development of the embryos is completed. The eggs deposited in the gelatinous matrix usually hatch as soon as the embryos are mature and thus can result in several generations each year.

UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

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ERADICANT FUNGICIDES OF POSSIBLE VALUE AGAINST THE AZALEA PETAL BLIGHT FUNGUS, OVULINIA AZALEAE

PHILIP BRIERLEY[1]

(Accepted for publication August 20, 1949)

Control of the petal blight disease of azalea, caused by *Ovulinia azaleae* Weiss, is now accomplished by use of protectant fungicides[2]. Before effective protectants became available, some tests were made of the possibility of eliminating, or at least reducing, the primary inoculum of ascospores by applying eradicant fungicides to the soil to prevent the development of the apothecial stage. However, two factors weigh against the practical use of eradicants in control of azalea petal blight, namely the hazard of injury by the fungicide to some of the diverse plants that accompany azaleas in Southeastern gardens, and the need for applications on a community basis in order to accomplish significant reduction of the disease. Protectants, therefore, have the more general appeal. The work on eradicants was discontinued in 1941, although two compounds, Cyanamid and Elgetol, had proved effective against the apothecia of *Ovulinia*. Since Plakidas[3] has recently suggested the study of eradicants, the data are presented here and may be of interest to workers who continue such studies.

Plots for experimental treatment near Charleston, South Carolina, were infested with blighted azalea flowers during the flowering season of 1940 (Tables 1 and 2) or were chosen in a heavily infested garden (Table 3). All plots were small because it was not practicable to count the tiny apothecia on large areas. Apothecia were first detected on February 28, and were common in the Charleston area by March 18, 1941. Few or none had developed in the test plots at the time the chemicals were applied, February 27 or March 12, 1941.

Cyanamid (calcium cyanamid) found effective against apothecia of *Sclerotinia fructicola* by Huber and Baur[4], and Elgetol (sodium dinitrocresylate), found toxic to perithecia of *Venturia* by Keitt and coworkers[5], were the compounds tested as eradicants. Elgetol was applied in water solution Cyanamid was tested in water suspension, but the grade used made a poor suspension. When not otherwise specified, the dry Cyanamid was diluted in white sand, which provided bulk and facilitated even distribution. The rates used were approximately those recommended by previous workers, or multiples of these rates.

[1] Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Beltsville, Maryland.

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In the first trial (Table 1), plots 6.5 ft. square (approximately 0.001 acre) were treated with two chemicals at three rates each in the spring of 1940. One azalea was set in each plot to test phytocidal action. Both Cyanamid and Elgetol burned the leaves with which they came in contact. These plots were covered with blighted flowers in April, 1940. During the summer the bushes were killed by flooding. On February 27, 1941, the treatments were repeated as shown in table 1.

TABLE 1.—*Ovulinia apothecia in 0.001-acre plots at Charleston, S. C., artificially infested with infected flowers in 1940. Treatments applied Feb. 27, 1941; apothecia counted March 18, 22, and 26, April 2 and 14, 1941*

Treatment		Number of apothecia	
Material	Amount applied per acre	Replication 1	2
Cyanamid, granular	200 lb.	0	0
	400 lb.	0	0
	800 lb.	0	0
Elgetol (1 per cent solution)	450 gal.	0	0
	900 gal.	0	0
	1800 gal.	0	0
Controls, untreated	.....	6	3
	.....	7	0
	.....	17	1

In the second trial, plots 2 × 2 ft. in an area lightly shaded by large trees were arranged in four rows of six each and heavily infested with blighted azalea flowers in April, 1940. Four chemical treatments were applied on March 12, 1941, each treatment appearing once in each of three blocks. Twelve untreated plots were randomized with the treatments in each block. The results (Table 2) indicate complete suppression of apothecia in all plots treated with either Cyanamid or Elgetol. The experiment was duplicated in an open field. Only one apothecium developed in the open, whereas 603 apothecia developed in the original experimental series that was shaded.

TABLE 2.—*Number of Ovulinia apothecia in 2 × 2-ft. experimental plots at Charleston, S. C., artificially infested with infected flowers in 1940. Treatments applied March 12, 1941; apothecia counted March 17, 22, and 25, April 2 and 12, 1941*

Treatment	Amount applied per acre	Block 1		Block 2		Block 3	
		Treated	Control	Treated	Control	Treated	Control
Cyanamid, granular	400 lb.	0	41	0	28	0	4
Cyanamid, powder	400 lb.	0	54	0	83	0	16
Cyanamid, powder (suspended in water)	400 lb.	0	68	0	107	0	12
Elgetol (1 per cent solution)	450 gal.	0	130	0	35	0	25
Totals		0	293	0	253	0	57

In the third trial, five replications of each of three cyanamid treatments were paired with untreated plots of equal size in an azalea garden in which flower spot had been consistently severe. As shown in table 3, natural infestation here was much lighter than the infestation artificially produced (Table 2). In two control plots no apothecia appeared, but two or more were found in each of 13 others, while no apothecia appeared in any of the 15 plots to which cyanamid was applied.

TABLE 3.—*Number of Ovulinia apothecia in paired 2×2-ft. plots in a naturally infested garden at Summerville, S. C. Treatments applied March 12, 1941; apothecia counted March 17, 22, and 25, April 3 and 10, 1941*

Treatment (400 lb. per acre)		Replication					Totals
		1	2	3	4	5	
Cyanamid, granular	Treated	0	0	0	0	0	0
	Control	6	3	12	15	8	44
Cyanamid, powder	Treated	0	0	0	0	0	0
	Control	9	6	0	5	0	20
Cyanamid, powder (suspended in water)	Treated	0	0	0	0	0	0
	Control	19	2	3	17	8	49

Preliminary trials were conducted to test the tolerance of azaleas to calcium cyanamide. On March 18, 1941, Cyanamid, 400 lb. per acre in sand, was applied to 5 plots at Magnolia Garden under old azalea bushes. The natural ground cover of light leaf mulch was undisturbed. On March 19, Cyanamid was applied at the same rate to five plots at Summerville, S. C., and to five at the Cypress Gardens. During the 1941 season no injury to azaleas was evident on any of the 15 plots. Apothecia, abundant in previous seasons, on some of the plots at Summerville were not detected after treatment. Higher amounts of Cyanamid, 800 and 1600 lb. per acre, produced no visible injury to azaleas in the season of application.

SUMMARY

Data presented indicate that treatment with Cyanamid or Elgetol in advance of apothecial development will suppress apothecia of *Ovulinia azaleae* for one flowering season. It is not known whether the sclerotia in treated areas are capable of developing apothecia in the following season. Unfortunately both the materials here shown to be effective against *Ovulinia* are injurious to the leaves of azaleas. Preliminary evidence indicates that Cyanamid is safe to apply to the soil about azaleas, producing no recognizable injury, at least in the season it is applied. No tests on the safety of applying Elgetol have been made.

BUREAU OF PLANT INDUSTRY
BELTSVILLE, MARYLAND

THE EFFECT OF ACTIDIONE ON THE GROWTH OF CERTAIN PATHOGENIC FUNGI AND ON THE GERMINATION OF PEA SEED[1]

V. R. Wallen, M. D. Sutton, and A. J. Skolko

(Accepted for publication August 22, 1949)

Actidione, an antibiotic product of the Upjohn Company of Kalamazoo, Michigan, isolated from cultures of *Streptomyces griseus*, has been reported by Felber and Hamner (1) to be capable of controlling powdery mildew of bean caused by *Erysiphe polygoni* D.C. when applied as a spray to affected plants at dilutions of 5 p.p.m. In view of this and earlier reports (2, 5, 6) on the antifungal effect of actidione at high dilutions on a number of yeasts and human pathogens, preliminary tests to determine its effect on certain seed-borne fungi and its possible application as a seed treatment were undertaken.

In the first test the agar streak method of antibiotic assay of Waksman and Reilly (4) was used and agar plates with actidione (crude product) dilutions of 1, 5, 10, 15, and 50 p.p.m. were prepared. Spore suspensions of the following species were streaked on Petri plates, as shown in figure 1: *Ascochyta pisi* Lib. and *Fusarium poae* (Pk.) Wollenw. on pea agar; *Colletotrichum lindemuthianum* (Sacc. & Magn.) Br. and Cav., *Alternaria brassicicola* (Schw.) Wilts. (= *Alternaria oleracea* Milbr.), *Helminthosporium sativum* P.K., and B., *Chaetomium globosum* Kze., and *Penicillium* sp. on malt-extract agar; and *Botrytis cinerea* Pers. on potato-dextrose agar.

The plates were incubated at room temperature (21°–24° C.) for 5 days. Examination of the plates disclosed wide variations in the effect of the actidione. In *Ascochyta pisi* (Fig. 1) and *Colletotrichum lindemuthianum*, growth was completely inhibited at a dilution of 1 p.p.m. In *Fusarium poae*, growth was sharply depressed at 1 p.p.m. and completely inhibited at 10 p.p.m. In *Alternaria brassicicola*, growth was retarded at 1 p.p.m., was evident only as a trace at 15 p.p.m., and was completely inhibited at 50 p.p.m. Growth of *Chaetomium globosum*, *Helminthosporium sativum*, and *Botrytis cinerea* (Fig. 2) was only slightly affected up to 15 p.p.m., but at 50 p.p.m. growth of all three fungi was strongly retarded though not wholly inhibited. In *Penicillium* sp., there was only a very slight reduction of growth even at 50 p.p.m. Though not unexpected, it was found that those species with thin-walled, light-colored spores were most readily affected by actidione.

Examination of hanging-drop mounts of the spores of *Alternaria brassicicola* and *Helminthosporium sativum* in various dilutions of actidione showed after 48 hr. that spore germination was sharply reduced at 15 p.p.m. and that, in those spores which had germinated, the germ tubes were ab-

[1] Contribution No. 989 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

The authors are indebted to Mr. J. E. Doyle, Plant Products Division, Production Service, Department of Agriculture, through whom the actidione was received.

normally short and swollen. In *Chaetomium globosum*, although total germination at 15 p.p.m. was only about half that in the check, germ tube development was much greater than in the check. This stimulatory effect was evident also in *Botrytis cinerea* where the percentage germination, as well as the germ tube length, was greater in the 15 p.p.m. dilution than in the check.

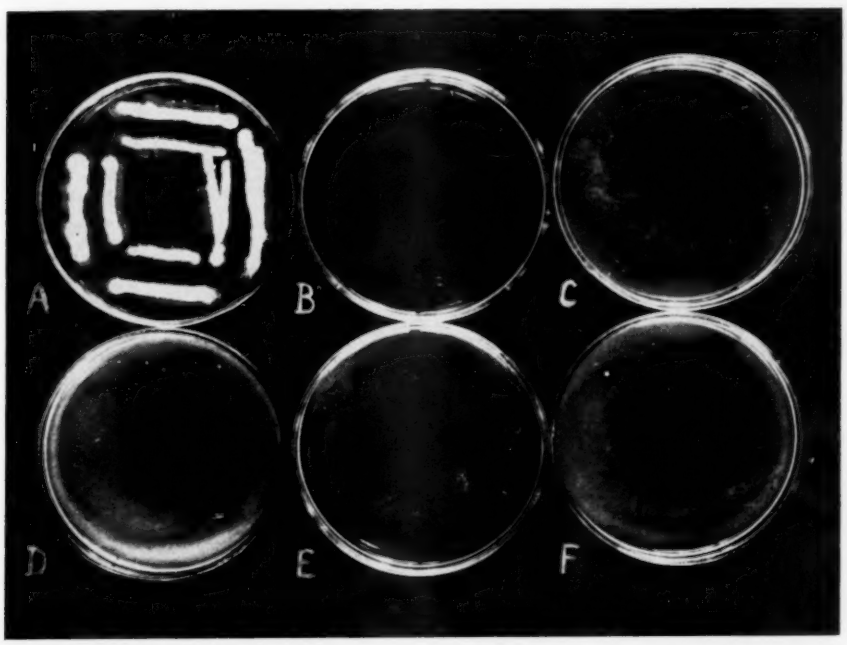

FIG. 1. Five-day-old plates of pea agar streaked with spore suspension of *Ascochyta pisi*. A, check; B, 1 p.p.m. actidione; C, 5 p.p.m.; D, 10 p.p.m.; E, 15 p.p.m.; F, 50 p.p.m.

In determining the effect of actidione on the growth of mycelial colonies of the fungi listed above, the filter paper disk method of Vincent and Vincent (3) was used. By this method, standard filter paper disks were saturated with the desired concentration of actidione and placed near the margin of the mycelial colonies on agar plates. With actidione dilutions up to 225 p.p.m., mycelial development was not affected at the point of contact between the disk and the fungus colony in any of the species or at any of the dilutions used. The mycelium grew over the disk without evidence of antagonism. It should be noted, however, that the time required for mycelial growth would permit diffusion from the disk and a concentration gradient would probably develop.

Although actidione failed to inhibit the mycelial growth of *Ascochyta pisi*, the effectiveness of actidione in inhibiting the germination and germ tube growth of spores of *A. pisi* in dilutions as high as 1 p.p.m. made it desirable to determine whether it was effective in controlling the pathogen

when internally borne in pea seeds. Five samples of 50 seeds each of Lincoln peas, which were known to be internally infected with *A. pisi* to the extent of 22 per cent, were treated with a 2 per cent chlorine solution for 15 min. to remove surface contamination. The samples were then soaked in actidione solutions of 1, 15, 40, and 225 p.p.m., and in sterile water as a check. After 24 hr. the seed was removed, plated on pea agar (5 seeds per Petri dish), and incubated at room temperature for 10 days. The percentage germination of the seed at the end of this period was 94 in the check and 84, 80, 72, and 52, respectively, in the 1, 15, 40, and 225 p.p.m. solutions of actidione. Bacterial contaminations were high in all treatments and

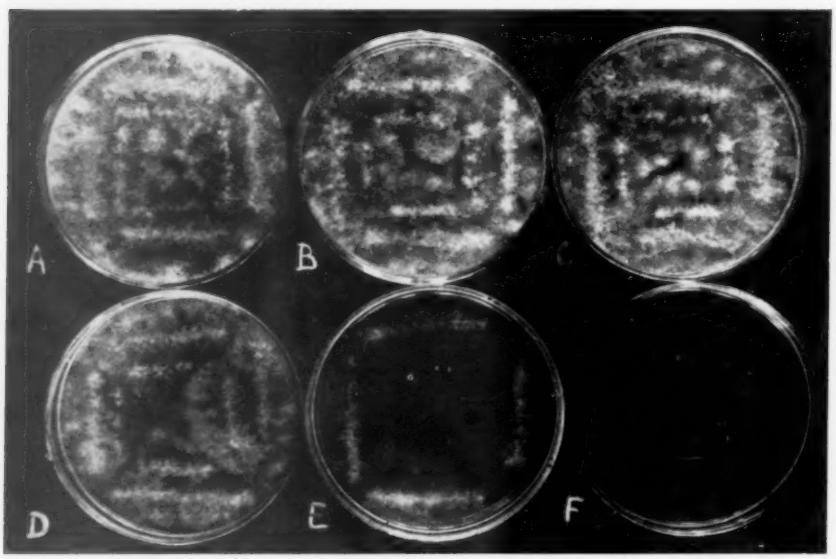

FIG. 2. Two-day-old plates of malt-extract agar streaked with spore suspension of *Botrytis cinerea*. A, check; B, 1 p.p.m. actidione; C, 5 p.p.m.; D, 10 p.p.m.; E, 15 p.p.m.; F, 50 p.p.m.

some seeds showed a white mycelial growth over their surface. Hyphal tip isolations from these mycelial colonies proved them to be *A. pisi*. It was apparent that actidione, even at concentrations as high as 225 p.p.m., failed to control internally-borne *A. pisi*, and that seed germination was reduced as the concentration of the actidione was increased.

Essentially similar results were obtained with Thomas Laxton peas, infected with *Ascochyta pisi* (49 per cent), that were treated similarly and planted in sterile soil in flats in the greenhouse. Emergence counts made at the end of 12 days showed 46 per cent emergence in untreated seed, 32 per cent emergence in seed soaked in sterile water for 24 hr., and 18, 4, 6, and 0 per cent emergence in seed soaked for 24 hr. in actidione solutions of 1, 15, 40, and 225 p.p.m. respectively. It would appear, therefore, that actidione not only failed to control internal *A. pisi* but actually ex-

erted a toxic effect on the pea seedling, as indicated by the marked reduction in germination.

The phytotoxic effect of actidione to young bean and oat seedlings at concentrations higher than 100 p.p.m. has been reported by Felber and Hamner (1). They found that at concentrations of 10 p.p.m. or lower there was no apparent effect on the bean plant at its various stages of development. No specific information on the effect on seed germination was given.

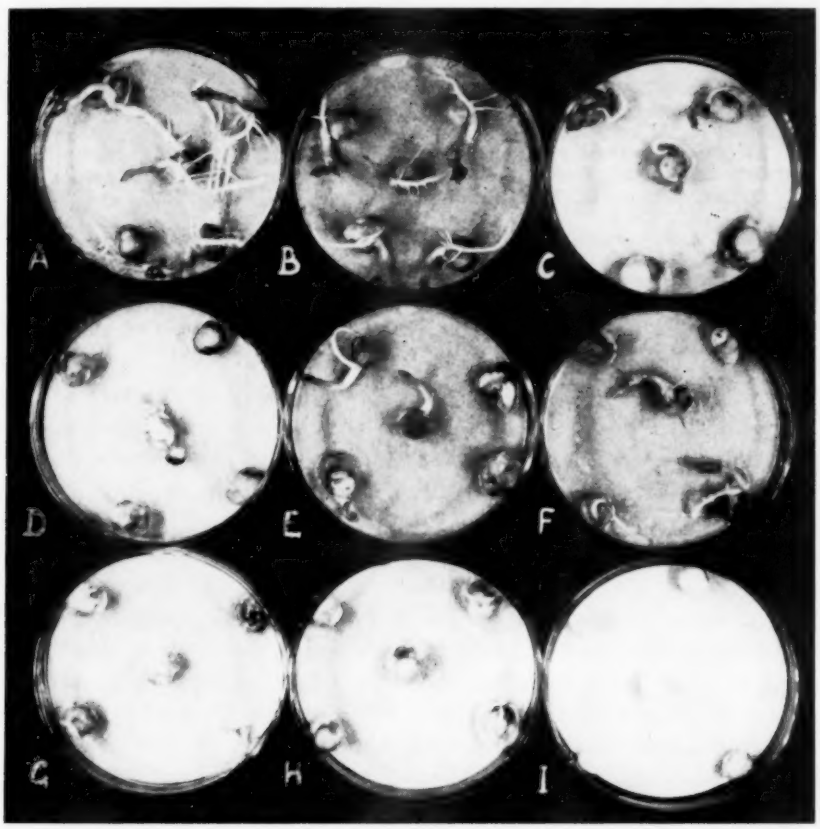

FIG. 3. Germination of pea seeds at 10 days on filter paper flooded with various dilutions of actidione. A, sterile water (check); B, 1 p.p.m.; C, 5 p.p.m.; D, 10 p.p.m.; E, 15 p.p.m.; F, 40 p.p.m.; G, 50 p.p.m.; H, 75 p.p.m.; I, 225 p.p.m. Note greatly increased shoot and reduced root development in B, C, and D; inhibition of germination of the seed and growth of saprophytic fungi in E–I.

To determine the effect of actidione on germinating pea seeds, filter papers in Petri plates were flooded with actidione dilutions of 1, 5, 10, 15, 40, 50, 75, and 225 p.p.m., and five seeds were placed in each plate. After 10 days some drastic effects of the actidione were observed (Fig. 3). Normally the pea seed germinates by producing a very long root and a relatively short epicotyl (as seen in the check). On the filter paper soaked in actidione of 1 p.p.m. dilution, the root system was abortive and discolored while the

shoot development was abnormally long and stout. This condition was even more accentuated at 5 p.p.m. At 10 p.p.m. and higher concentrations, germination was almost entirely inhibited. Equally striking was the abundant growth of saprophytic fungi and bacteria at the high concentrations, which was probably stimulated by the death of the cotyledonary tissue.

DIVISION OF BOTANY AND PLANT PATHOLOGY
SCIENCE SERVICE
OTTAWA, CANADA

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EFFECT OF HOST VARIETY AND OTHER FACTORS ON PATHOGENICITY OF POTATO RING-ROT BACTERIA

REINER BONDE AND MILDRED COVELL[1]

(Accepted for publication August 30, 1949)

INTRODUCTION

Studies have been conducted in Maine to develop potato varieties resistant to the bacterial ring-rot disease caused by *Corynebacterium sepedonicum* (Spieck. and Kott.) Skap. and Burk. (1 through 7). These studies showed that a number of resistant seedling and commercial varieties became infected with ring rot to a much lesser degree than did the commonly grown susceptible varieties, even when subjected to rather drastic inoculation methods. It seemed of value to know whether the resistant varieties would contract the disease in higher percentages if inoculated by still more severe methods.

It has been stated orally that the resistant varieties, developed as a result of the National Potato Breeding Program, are not actually resistant to infection by the ring-rot bacteria, but are highly tolerant and may be symptomless carriers of the disease. Varieties that harbor the bacteria without showing symptoms would serve to perpetuate and disseminate ring rot and so would nullify nongenetic control measures. A study therefore was made to determine whether certain resistant varieties may be symptomless carriers of potato ring rot.

There also is the possibility that more virulent strains of ring-rot bacteria will arise as resistant potato varieties are developed. This was studied also.

In this paper a distinction must be made between infected plants or tubers that are obviously diseased—that is, with macroscopic symptoms—and those that are apparently healthy (judging from macroscopic appearance) but bacteria-harboring as disclosed by infection experiments or microscopic examination. The apparently healthy plants and tubers may be bacteria-harboring or bacteria-free. Resistance as considered in this paper is relative, immunity to bacterial ring rot not yet having been found in potatoes.

COMPARISON OF DIFFERENT METHODS OF INOCULATION

Pure cultures of the ring-rot organism, when grown on artificial media, have not given reliable and consistent inoculation results in Maine. Therefore, the bacteria from obviously diseased portions of tubers were used as the inoculum in infection studies. The first such inoculations were made by rubbing or smearing the bacterial ooze from Katahdin tubers onto freshly cut surfaces of potato seed pieces just prior to planting. Later experiments showed that consistently higher percentages of infection occurred if the

[1] Plant Pathologist and Technical Assistant, respectively, Maine Agricultural Experiment Station.

freshly cut seed pieces were dipped into a heavy suspension of the bacteria, or if a water suspension of the bacteria was pricked into the eyes of the seed pieces.

Table 1 compares these two more infective methods of inoculation on the susceptible control varieties Katahdin and Green Mountain and the

TABLE 1.—*Percentage ring-rot infection in potato plants and tubers in 14 resistant varieties inoculated by two methods. Katahdin and Green Mountain included for comparison*[a]

Variety or selection number	Parentage[c]	Infection from inoculating freshly cut seed pieces by two methods[b]			
		Dipped in bacterial suspension		Bacterial suspension pricked into eyes	
		Plants	Tubers	Plants	Tubers
		Pct.	*Pct.*	*Pct.*	*Pct.*
Resistant					
824	Houma × 336–144	10.0	2.8	0.0	0.0
826	Do	20.0	1.1	20.0	0.0[d]
848	Sebago × 336–144	6.0	1.0	0.0	0.0
854	Do	0.0	0.0	0.0	0.0
870	Earlaine × 336–144	7.5	1.8	0.0	0.0
886	Do	25.0	6.1	40.0	0.0[d]
895	Do	0.0	0.0	0.0	0.0
899	336–144 × Earlaine	0.0	0.0	20.0	0.0[d]
903	Do	0.0	0.0	0.0	0.0
915	Do	15.0	6.3	20.0	0.0[d]
917	Do	0.0	0.0	20.0	0.0[d]
1162	41956 × 336–144	0.0	0.0	20.0	0.0[d]
Teton	45146 × Earlaine	0.0	0.0	20.0	0.0[d]
President	Richter's Imperator × Wilhelm Korn	0.0	0.0	40.0	40.0[e]
Susceptible controls					
Katahdin	40568 × 24642	65.0	70.0	80.0	80.0
Green Mountain	Dunmore × Excelsior	100.0	80.0	80.0	80.0

[a] In this experiment none of the resistant varieties became infected when inoculated by rubbing or smearing the bacterial ooze into the freshly cut surfaces of potato seed pieces just prior to planting, compared with 70 and 82.3 per cent, respectively, for the susceptible Katahdin and Green Mountain varieties.

[b] Based on 100 inoculated seed pieces for each method of inoculation in each variety, with sometimes a few lost. Tubers from the plants examined for infection at harvest time and after 8 weeks in storage.

[c] Seedling 336–144 is a resistant variety developed by crossing President with Katahdin. It was later named Saranac.

[d] Not obviously infected, although from the obviously infected plants.

[e] No active decay in tubers after being in storage. Bacteria in vascular tissue revealed on microscopic examination.

resistant varieties President and 13 selected seedlings. Six of the 14 resistant varieties became infected when inoculated by dipping the seed pieces in a suspension of the bacteria. However, the percentage of plants and tubers that became diseased was relatively small in comparison with that which occurred in the Katahdin and Green Mountain controls.

Pricking the inoculum into eyes of the potato seed pieces was a somewhat more effective method of inoculation: eight of the 14 resistant seedlings

and varieties developed diseased plants; moreover, there was a tendency toward a higher percentage of obviously diseased plants. However, it should be observed that—with the exception of the variety President—when the pricking method was used no obvious infection was found in the tubers of resistant varieties, even though some of the plants showed symptoms of the disease.

Another experiment was conducted for the purpose of determining further the most effective method of inoculating potato seed pieces, and whether the resistant variety Teton would be more subject to infection when inoculated by the more successful methods.

The percentages of obvious infection were compared in the Teton and Katahdin varieties inoculated by four different methods (Table 2). The

TABLE 2.—*Comparison of infection in Teton and Katahdin varieties inoculated by four different methods*

Method of inoculating seed pieces	Average infection resulting[a, b]	
	Teton	Katahdin
	Percent	*Percent*
Inoculum pricked into eyes with sterile needle	2.4	100.0
Freshly cut and dipped into heavy suspension of bacteria	0.4	100.0
Inoculum rubbed into eyes and small growing sprouts	3.7	99.6
Freshly cut surfaces smeared with bacteria taken directly from infected tubers	0.0	91.6*
All methods of inoculation combined	1.1	99.2**

* Significantly different from other methods at the 5 per cent level.
** Significantly different from Teton at the 1 per cent level.

Analysis of variance of transformed data

Source of variation	Degrees of freedom	Mean square	F
Inoculation	3	358.5	3.8*
Varieties	1	62,297.1	664.1**
Replicates	4	33.2	0.4
Treatment *vs.* variety	3	88.1	0.9
Error	28	93.8	

* Significant at 5 per cent level.
** Significant at 1 per cent level.
[a] Average from five replicated 10-hill plots for each treatment in each variety.
[b] Percentages of infection for data in tables 2, 4, 5, 6, and 7 were transformed to angles in degrees for analysis of variance. Since the average percentages of infection are obtained by reconverting from angles, these means will deviate somewhat from the ordinary arithmetical average. Tests of significance were applied to transformed means, therefore no L.S.D. values appear in tables. See Snedecor (10, pp. 447–448), and Hayes and Immer (8, pp. 375–379).

analyses show that three methods of inoculation, namely, 1) inoculum pricked into eyes of seed pieces with a sterile needle, 2) dipping the freshly cut seed pieces in a heavy suspension of the bacteria, and 3) rubbing the inoculum into the eyes and small growing sprouts, were about equally effective. The poorest method was that of smearing the freshly cut surfaces of the seed

pieces with bacteria taken directly from infected tubers, in which case the percentage of infection was significantly lower. When the percentages of infection in the two varieties were analyzed separately, it was found that in the Katahdin variety, the smearing method resulted in significantly less infection. The average percentage of infection when all of four inoculation treatments were combined was 1.1 per cent for Teton, and 99.2 per cent for Katahdin.

COMPARATIVE PATHOGENICITY OF BACTERIA FROM RESISTANT AND SUSCEPTIBLE VARIETIES OF POTATOES

First Experiment

The question naturally has arisen as to whether biological races of *Corynebacterium sepedonicum* with higher pathogenicity will be formed as resistant varieties of potatoes are developed. It is well known that a variety often loses its resistance to infection when more virulent strains of the pathogen are developed in nature or are introduced from other localities (11).

Starr and Riedl (12), working in Wyoming, found that ring-rot bacteria from the resistant variety Teton were no more pathogenic than those from Bliss Triumph and other susceptible potato varieties. For seven successive years the writers have inoculated Teton and other resistant varieties with the bacteria from infected tubers of the susceptible Katahdin variety. The resistant varieties have continued to show a marked degree of resistance and in most cases no obvious infection, or a relatively small amount, resulted. In 1944, however, 18 per cent of inoculated plants of the Teton variety became infected (6, table 1). The cause of such occasional, relatively high increases in the amount of obvious infection in a resistant variety like Teton is not known.

A number of experiments were conducted for the purpose of determining whether ring-rot bacteria from the resistant variety Teton are more pathogenic than those from susceptible varieties. In the following experiments the potatoes were inoculated by dipping in a bacterial suspension.

In 1946, bacteria from Teton, S.055, and Katahdin varieties, respectively, were used as inoculum and their relative pathogenicities were compared on the same varieties (Table 3). Although the data are too few for a satisfactory statistical analysis, a highly significant difference appeared in percentage of obvious infection between the two susceptible varieties, S.055 and Katahdin (80.5 per cent and 97.8 per cent of inoculated plants, respectively), and the resistant variety Teton (4.3 per cent). Also, S.055 apparently tended to be more resistant than Katahdin. There was no significant difference in infectivity of the inocula.

Second Experiment

Another experiment was conducted for the purpose of determining whether ring-rot bacteria from obviously infected Teton tubers (resistant)

are more pathogenic than those from similar Katahdin tubers (susceptible). For this experiment the percentages of obvious infection that occurred in the Teton and Katahdin varieties were compared when each was inoculated with bacteria from obviously infected tubers of both varieties. Comparative

TABLE 3.—*Comparison of percentages of infection resulting from inoculations with bacteria from resistant and susceptible varieties*

Source of inoculum	Variety inoculated[a]	Ring-rot infection resulting[b]
		Per cent
Teton	Teton	6.4
	S 055	97.0
	Katahdin	96.0
S.055	Teton	2.6
	S 055	69.6
	Katahdin	97.5
Katahdin	Teton	4.0
	S.055	75.0
	Katahdin	100.0
All sources combined	Teton	4.3
	S 055	80.5**
	Katahdin	97.8**

[a] Under field conditions Teton is very resistant to infection, S.055 possesses some resistance, and Katahdin is very susceptible.
[b] Mean from 100 inoculated seed pieces for each source of inoculum in each variety. Infection determined in plants and in tubers from inoculated plants.
**Significantly different from Teton at 1 per cent level.

inoculations also were made with inoculum from apparently healthy tubers from inoculated plants of these two varieties to determine whether some of such tubers also were infected. Table 4 summarizes the results secured from this experiment.

TABLE 4.—*Comparison of pathogenicity of bacteria from Teton and Katahdin varieties*

Source of inoculum	Ring rot in inoculated plants[a, b]	
	Teton	Katahdin
	Per cent	*Per cent*
Teton tubers with symptoms	17.7**	99.4*
Katahdin tubers with symptoms	1.8	93.2
Symptomless tubers from inoculated Teton plants	0.0	0.0
Symptomless tubers from inoculated Katahdin plants	1.2	5.5

[a] Average of seven replicated 20-plant plots for each treatment.
[b] See footnote b, table 2.
* Significantly different from Katahdin at 5 per cent level.
** Significantly different from Katahdin at 1 per cent level.

By analysis of variance, the data show that the bacteria developed in the obviously infected tubers of the resistant Teton variety were significantly more pathogenic than those produced in similar tubers of the susceptible

Katahdin variety. The differences in the virulence of the two kinds of inoculum are shown by the percentage of ring rot given in the upper part of table 4. The bacteria from the obviously infected Katahdin tubers produced only 1.8 per cent diseased plants when used to inoculate the Teton variety. In contrast, the inoculum from the obviously infected Teton tubers caused 17.7 per cent or 9.8 times as much infection.

It also can be stated that the Teton inoculum was significantly more virulent than the Katahdin inoculum when used to infect the susceptible Katahdin variety, causing 99.4 per cent infection compared with 93.2 per cent infection for Katahdin inoculum.

The present writers' data are somewhat contrary to those secured in Wyoming where the bacterial ooze from the susceptible variety Bliss Triumph caused more severe plant symptoms and more numerous bacteria in the infected tissue than did the ooze from the resistant Teton variety (9). It was concluded in Wyoming that the ring-rot bacteria infecting the Teton variety and ring-rot-resistant seedlings were not more pathogenic than those carried by susceptible varieties such as Bliss Triumph (12).

TEST OF VARIETIES AS MASKED CARRIERS OF RING-ROT BACTERIA

The question has arisen whether the apparently healthy tubers from inoculated resistant plants are free of infection. Infected but symptomless tubers could become additional sources of infection and thus help to disseminate the disease.

The experimental results, as shown in the lower half of table 4, indicate that Teton is less likely to be a masked carrier of ring rot than the Katahdin variety. The Teton inoculum from symptomless tubers produced no ring-rot infection in either variety. In contrast, the Katahdin inoculum, also from apparently healthy tubers, produced infection in both varieties, showing that the bacteria were present although symptoms were not apparent. However, it has been observed in other experiments that apparently healthy tubers from inoculated Teton plants are not always entirely free from the disease.

The greater susceptibility to ring-rot infection by Katahdin in comparison with Teton is further shown in table 5, using the same original data as

TABLE 5.—*Comparison of ring-rot infection in Teton and Katahdin varieties, using inoculum from both varieties combined*

Variety inoculated	Ring rot resulting[a] with inoculum from	
	Obviously diseased tubers	Apparently healthy tubers
	Per cent	*Per cent*
Teton	7.9	0.3
Katahdin	97.1**	1.4

[a] See footnote b, table 2.
** Significantly more susceptible than Teton variety at 1 per cent level.

for table 4. The varietal response to infection by the inoculum from obviously diseased tubers of both varieties combined differed significantly in degree of infection at the 1 per cent level.

The difference between Teton and Katahdin inoculum in percentage of ring-rot infection that resulted, when each was secured in turn from obviously diseased and then from apparently healthy tubers, may be observed further in table 6, using again the same data as in table 4.

TABLE 6.—*Comparison of pathogenicity of ring-rot bacteria from obviously diseased and apparently healthy tubers from inoculated Teton and Katahdin plants. Infection in both varieties combined*

Source of inoculum	Ring-rot infection[a, b]
	Per cent
Teton—obviously diseased tubers	67.4
Teton—apparently healthy tubers from inoculated plants	0.0
Katahdin—obviously diseased tubers	43.6
Katahdin—apparently healthy tubers from inoculated plants	3.0

Analysis of variance, percentage data transformed to degrees

Source of variation	Degrees of freedom	Mean square	F
Total	55		
Varieties	1	15,942	253**
Obviously diseased and apparently healthy tubers	1	26,242	417**
Sources of inoculum: Teton and Katahdin	1	55	
Varieties vs. obviously diseased and apparently healthy tubers	1	12,727	202**
Varieties vs. Teton and Katahdin inoculum	1	164	2.6
Teton and Katahdin inoculum vs. obviously diseased and apparently healthy tubers	1	1,984	31**
Replications	6	53	
Error	43	63	

[a] Average percentage infection from seven replicated plots of 20 plants each for treatment in each variety.

[b] See footnote b, table 2.

**Highly significant at 1 per cent level.

The inoculum from the obviously diseased Teton tubers resulted in 67.4 per cent infection, and that from the apparently healthy tubers of the same variety produced no infection. The Katahdin inoculum from the obviously diseased and apparently healthy tubers resulted in 43.6 and 3.0 per cent infection, respectively. The interaction, which is highly significant, shows a differential response of the Teton and Katahdin inoculum when each is obtained from obviously diseased and apparently healthy tubers from inoculated plants.

Since no infection resulted when the apparently healthy Teton tubers were used as a source of inoculum, the data fail to show the Teton variety to be a symptomless carrier of ring-rot bacteria. This fact is very important in respect to the development of varieties of potatoes that are resistant

to or immune from the ring-rot disease. In considering all data obtained by the writers, it can be stated that, although the pathogen may perhaps sometimes be present in apparently healthy Teton tubers, this and some other resistant varieties are less likely to be carriers of the disease than are the Katahdin and some other commercially grown susceptible varieties.

In 1946 the writers harvested the tubers from 50 previously inoculated plants of the Teton and Katahdin varieties. The apparently healthy tubers from the inoculated hills of both varieties were planted in 1947 and the progeny of these were replanted, without reinoculation, in 1948. All of the Teton hill lots selected as being healthy produced plants with no infection found in either 1947 or 1948, even when examinations were made with the aid of the microscope. In contrast, only two hill lots of the Katahdin variety failed to show signs of the disease. Some of the Katahdin hill lots which did not show ring-rot symptoms in 1947 did so in 1948 and it is possible the two remaining hill lots also were infected in trace amounts. Therefore the disease may be present in the Katahdin variety and symptoms may not appear in either tubers or foliage for at least one year. These data help to explain the reason farmers have found it very difficult or even impossible to eradicate ring rot from infected seed stocks.

The writers' experiments also have shown that while Katahdin plants infected with ring-rot-contaminated water had no symptoms in the foliage or tubers during the growing season or when the seed tubers were planted the following spring, the symptoms were present when the crop was harvested the following fall. Thus there was an incubation period of over a year.

Hill (9) reports that there is evidence from studies conducted in Wyoming that the ring-rot disease may be present in potatoes for several years without visible symptoms.

In some previously reported experiments the same seed stocks of President, Teton, S.824, and S.870 were inoculated for four successive years with no appreciable increase in the amount of the disease (7, table 1). The seed stocks of the susceptible varieties Green Mountain, Houma, and Katahdin were eliminated after one year because of the high percentages of infection.

COMPARISON OF INFECTED TETON, KATAHDIN, AND GREEN MOUNTAIN TUBERS AS TO AMOUNT OF ROT

It has been observed that varieties differ in the amount of decay occurring in tubers infected with ring rot. The infected Green Mountain tuber is very susceptible to decay and in Maine it was difficult to maintain infected seed stocks of this variety in storage. The infected Katahdin tuber is less susceptible to decay and therefore Katahdins have been used extensively when relatively large quantities of diseased material were needed for infection and inoculation studies.

In 1946 the tubers from 100 inoculated plants in each of the varieties

Teton, Katahdin, and Green Mountain were placed in storage. The following spring there was less than 3 per cent of decayed tubers in the Teton variety and the crop was practically all edible. Many of the Katahdin tubers had rotted and much of the crop was unfit for the market, while some bacteria-harboring tubers were still firm and intact. The Green Mountain tubers, in contrast, were very badly decayed and the entire crop was unsalable.

Twenty infected hills of the Teton and Katahdin varieties were harvested separately in 1946 and their tubers were placed in storage until the following spring. The relative amounts of tuber rot found in the two varieties are summarized in table 7. There was significantly more tuber decay in the

TABLE 7.—*Comparison of percentage of tuber decay from infected Teton and Katahdin plants and in progeny from apparently healthy tubers*

Variety	Percentage active decay[a] in tubers of	
	Infected plants[b]	Progeny from apparently healthy tubers[c]
Teton	29.8	0 04
Katahdin	84.3**	44.2**

[a] See footnote b, table 2.
[b] Decay in tubers from inoculated plants known to be infected.
[c] Decay in progeny from apparently healthy tubers which were replanted.
**Significantly higher than Teton at the 1 per cent level.

Katahdin variety (84.3 per cent) than in the Teton variety (29.8 per cent).

Table 7 also shows the percentage of infected tubers that resulted when the apparently healthy tubers from the inoculated plants were replanted. Approximately 44 per cent of the tuber progeny of the plants produced by the apparently healthy Katahdin seed tubers were infected at the time of harvest. The tuber progeny of the plants grown from apparently healthy Teton seed tubers from inoculated plants showed only a trace of tuber rot.

NO NATURAL RECOVERY FROM INFECTION IN RESISTANT VARIETIES

Unpublished field studies in Maine have indicated that some resistant varieties lose their ring-rot infection naturally on being perpetuated in the field. To test this idea, obviously infected tubers from 56 resistant and tolerant seedlings were selected and planted in the field for observation. The progenies from all of these infected seed stocks developed symptoms of the disease.

DISCUSSION

A number of seedling varieties of potatoes, developed by the personnel of the National Potato Breeding Program, are highly resistant to ring-rot infection. Studies were conducted to determine whether the resistant varieties would become infected to a higher degree when more drastic methods of inoculation were used.

Contamination of the freshly cut surfaces by the seed-cutting knife and introducing the bacteria into tuber wounds in the process of handling and storing the crop are two of the chief means of disseminating ring rot in Maine. The results presented, however, show that contamination of freshly cut seed pieces by the smearing method is an ineffective method of inoculating resistant varieties. Dipping freshly cut seed pieces in a suspension of the bacteria and pricking a suspension of the bacteria into the eyes produced significantly more infection than smearing freshly cut surfaces of seed pieces. Relatively low percentages of infection resulted in the resistant varieties even when inoculated by the more drastic methods.

Rubbing the inoculum into the eyes and the young sprouts of the seed pieces also caused a significantly higher percentage of infection than smearing the cut surfaces. This fact has an important bearing on the dissemination of ring rot in Maine, because the seed tubers, at the time of planting, generally are slightly sprouted and the inoculum is very effective under such conditions. Here again, however, the resistant Teton variety had very little infection in comparison with Katahdin, even with this severe method of inoculation.

The data presented are based on artificial inoculations which were much more severe than the contamination that may occur normally in the process of handling and storing the crop by the farmers. It is concluded that under natural field and storage conditions very little or no infection would occur in a resistant variety like Teton.

Workers in Wyoming concluded that the bacteria from the resistant variety Teton were not more pathogenic than those carried in susceptible varieties like Bliss Triumph. The Maine studies, however, showed the bacteria from resistant Tetons may be more virulent than those in susceptible Katahdins. This supports the opinion of some research workers that the resistant varieties developed will be susceptible to infection when more virulent forms of bacteria are produced in nature. However, Teton and some other resistant varieties have been grown in experimental plots in Maine for seven or more years, with no apparent loss in resistance. Although the bacteria from Teton were more pathogenic than those from Katahdin, relatively little infection occurred in the resistant varieties, even when the more virulent bacteria were used for inoculation.

The resistant seedling varieties that have been developed are not immune to the disease. The symptoms often are indistinct and the plants are rather tolerant. It may be justly asked whether such resistant varieties will not actually be carriers of the disease and serve as a continued source of infection. The studies presented indicate that the Teton is less likely to be a carrier than the susceptible Katahdin.

The writers found that the ring-rot bacteria may be present, undetected, for a year or more in apparently healthy Katahdin seed stocks, and they failed to eliminate the disease from an infected Katahdin seed stock. In contrast, a healthy Teton seed stock was developed under similar conditions.

This may explain the reason why farmers have found it very difficult to eliminate ring rot from infected seed stocks of susceptible varieties. It may be possible for farmers to eradicate the disease from a resistant variety and then maintain it free from disease for a long time.

Decayed tubers constitute one of the serious losses resulting from ring-rot infection. Some growers in Maine have discarded from 30 to 85 bushels of rotted potatoes per acre and in some cases the contents of entire bins were badly decayed from ring rot. Infected Teton plants had significantly less tuber decay than did those of Katahdin. Furthermore, there was very little decay in the progeny when tubers from infected Teton plants were replanted. In view of this fact, it seems that farmers would lose very little of their crop from ring-rot tuber decay if Teton or other resistant varieties were grown.

SUMMARY

Three different methods of inoculation with the ring-rot bacteria *Corynebacterium sepedonicum* (Spieck. and Kott.) Skap. and Burk. were compared on susceptible Katahdin and Green Mountain controls and the resistant variety President and 13 selected resistant potato seedlings. None of the resistant varieties became infected when inoculated by smearing of seed pieces with bacterial ooze from obviously diseased tubers. The Katahdin and Green Mountain controls were infected 70.0 and 82.3 per cent, respectively, under the same conditions.

Dipping freshly cut seed pieces in a suspension of the bacteria, pricking the suspension of bacteria into the eyes of seed pieces, or rubbing the inoculum into eyes and sprouts were effective methods of inoculation. Relatively small percentages of infection occurred in the resistant varieties when inoculated by the severe methods.

There was a striking difference in percentage of ring rot in Teton and Katahdin varieties when inoculated by artificial methods. The average percentage infection for one experiment, when all inoculation treatments were combined, was 1.1 per cent for Teton and 99.2 per cent for Katahdin.

The ring-rot bacteria from obviously infected tubers of the resistant Teton variety were more pathogenic than those from the susceptible Katahdin variety.

The experimental results indicate that the resistant Teton is less likely to be a masked carrier of ring rot than Katahdin.

Ring-rot infected plants of the Teton variety had significantly less tuber decay than did infected plants of the Katahdin variety.

MAINE AGRICULTURAL EXPERIMENT STATION
ORONO, MAINE

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EFFECT OF TEMPERATURE ON THE FUNGICIDAL ACTION OF SULPHUR

C. E. Yarwood

(Accepted for publication August 30, 1949)

It has been commonly asserted that the fungicidal activity of sulphur increases with increasing temperature, but consistent and quantitative data in support of this assertion are hard to find. Boureart (2) reports that sulphur kills "fungi living as parasites on plants" in 1 to 3 days at 30 to 40° C., but requires 4 to 5 days at 25 to 30° C. and longer at 25° C. No experimental or observational basis for this statement is indicated. Doran (4) presents data on spore germination of various fungi which show that sulphur is more effective at 20° and 26° C. than at 10° C., but these data do not show the relative activity at these or other temperatures. Bioletti (1) says sulphur is effective against grape powdery mildew only at about 75° F. and above, but it is not clear that any quantitative observations were made. Mares (7) states that temperatures of 20° C. and above are necessary for the lethal action of sulphur against grape powdery mildew. Smith (14) reports that sulphur was effective in controlling brown rot of peaches at 40°, 45°, and 55° F., but not at temperatures above 65° F. Yarwood (16) indicates that sulphur dust was effective in controlling hop downy mildew at temperatures as low as 4° C., and in controlling snapdragon rust at 7° C. Such fragmentary and apparently contradictory reports indicate the need for a quantitative study of the fungicidal action of sulphur over a range of temperatures. Such has been supplied by Horsfall (5) and McClellan (9), and calculations from their data are presented in figure 4. In both cases the relative percentage inhibition of spore germination due to sulphur is plotted against temperature. The data of McCallan and Wilcoxon (8) for evolution of hydrogen sulphide from sulphur by *Botrytis cinerea* may not be closely applicable here, but are presented. The results obtained by these four investigators, which are the only quantitative data the writer has been able to find on the subject at hand, do not appear to the writer to indicate a rational relation between temperature and the fungicidal activity of sulphur.

The relation of temperature to the vapor pressure and rate of volatilization of sulphur may have an important bearing on its fungicidal action. Data on the vapor pressure of sulphur over the range of temperatures used in these studies have not been found, but the vapor pressure of sulphur as derived by extrapolation from the data of Menzies (10) is presented in figure 4. This curve is not corrected for the known decrease in the temperature coefficient with increasing temperature, but this lack does not introduce any errors of apparent importance here. Data on volatility of sulphur from the work of Liming (6) and Tucker (15) are also presented for comparison, but other investigators' data are not used because, in the

cases which have come to the author's attention, they refer almost exclusively to temperatures above those of this study.

METHODS

The writer has attempted by several basically different methods to determine the relation of temperature to the fungicidal action of sulphur. Only the methods described below with bean powdery mildew (*Erysiphe polygoni* D. C.) and bean rust (*Uromyces phaseoli* (Pers.) Wint.), both of which diseases are effectively controlled commercially with sulphur, appear worthy of report at this time.

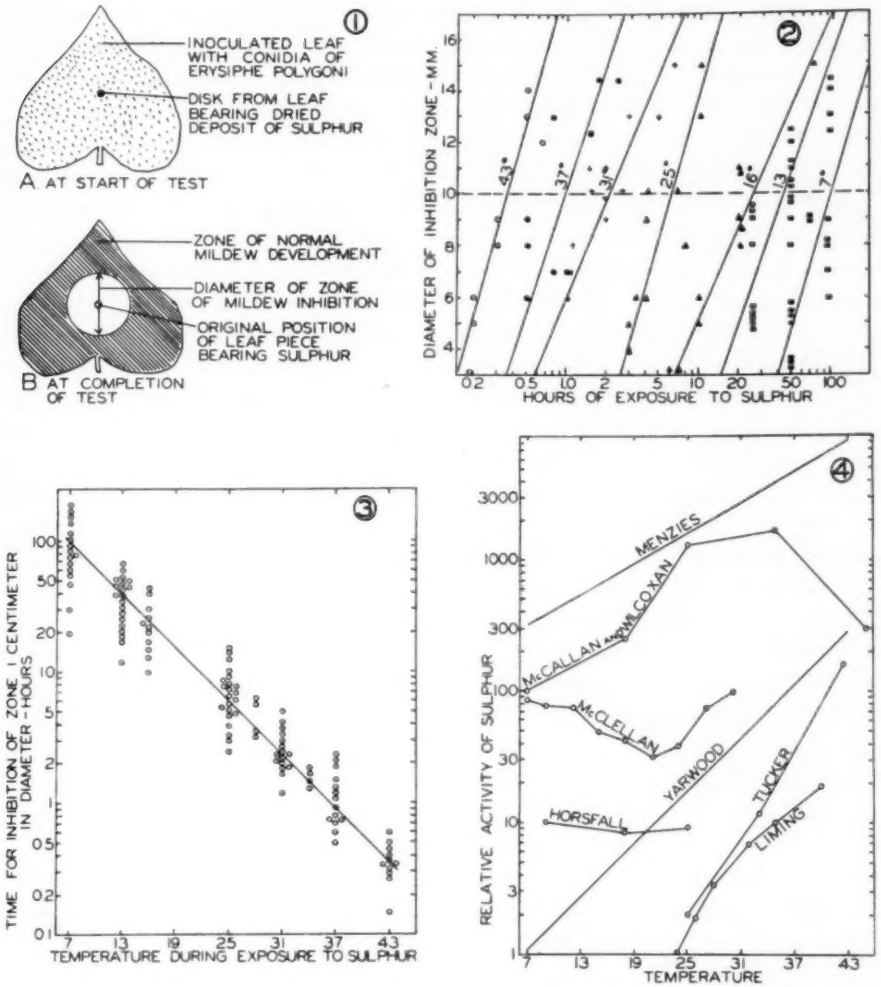
Using bean powdery mildew, disks of 3.5-mm. diameter, cut from living bean leaves sprayed about 1 hr. previously on both surfaces with 10 per cent lime sulphur plus spreader, were placed on the upper surface of mildew-inoculated detached bean leaves on 8 per cent sucrose, in Petri dishes. The inoculated leaves, with disks bearing the dried deposit of sulphur, were placed at a range of test temperatures in the dark for a range of test intervals. The disks bearing the sulphur deposit were then removed and the leaves on sugar solution were incubated in diffuse daylight at about 24° C. The growth of mildew was inhibited in a circular zone beyond the original position of the sulphur disk (Fig. 1). The diameter of the zone of mildew inhibition was measured with a millimeter scale after the leaves had incubated 4 days at 24° C.

A number of variations in the technique were tried. In some tests the leaf disks bearing the lime-sulphur deposit were in alternate units held about 1 mm. above the inoculated leaf surface by means of a cheesecloth screen. For inhibition zones over 3 mm. in diameter, no consistent difference in the amount of mildew inhibition was observed to depend on whether or not the disk bearing sulphur was directly on the inoculated leaf surface, or was held above the inoculated leaf by means of a cheesecloth screen. The inhibition zones were obviously due to gaseous diffusion of the sulphur and did not depend on any contact or surface effect.

The time of measurement of the inhibition zone was important. This zone was greatest when first apparent and gradually decreased in size, presumably principally because of the lateral intrusion of the mildew mycelium, but perhaps also because of the delaying rather than lethal effect of the sulphur on the mildew at the margin of the inhibition zone. In 60 pairs of successive measurements of the same inhibition zones made 1 to 3 days apart, the decrease per day in the diameter of the inhibition zones ranged from 0 to 3 mm. and averaged 1.2 mm.

In trials of the application of lime sulphur with and without a spreader to the leaves from which the disks were obtained, the spray deposit appeared more uniform when a spreader was added. Therefore 0.1 per cent phthalic glycerol alkyd resin was used throughout.

Elemental sulphur with spreader was tested as a spray for sulphur deposit. The dried deposit from an application of spray containing 3 per



FIGS. 1–4. Relation of temperature (in degrees centigrade) to the fungicidal action of sulphur for bean powdery mildew.

FIG. 1. A. Diagram of mildew-inoculated bean leaf with leaf disk bearing sulphur deposit in place. B. Diagram of mildew-infected bean leaf showing the zone of inhibition of mildew around the original position of the leaf disk bearing the sulphur deposit.

FIG. 2. Relation of time of exposure to sulphur to the diameter of mildew-inhibition zones at different temperatures. Because they would overlap with temperature numerals or because they were beyond the scale limits chosen, some values have been omitted.

FIG. 3. The relation of temperature of exposure to sulphur to the time for inhibition of a mildew zone 1 cm. in diameter.

FIG. 4. The relation of temperature to the activity of sulphur as reported by different investigators. The various graphs are vertically spaced for convenience of observation, and the vertical distances between graphs have no significance. Comparison between different graphs should be made with respect to slope only. The different graphs may be converted to absolute numerical values as follows:

Menzies: vapor pressure at 43° C. = 0.000083 mm. mercury.

McCallan and Wilcoxon: at 35° C. amount of hydrogen sulphide = 17 mg.

McClellan: at 30° C. reduction in germination due to sulphur = 65 per cent.

Horsfall: at 9° C. reduction in germination due to sulphur = 85 per cent.

Tucker: at 42.5° C. sublimation rate = 8.4 mg./cm.^{2}/24 hr.

Liming: at 40° C. sulphur tarnish formed in 16.8 hr.

Yarwood: at 43° C. inhibition of 1-cm.-diameter area of mildew occurred in 21 min. (Fig. 3).

cent elemental sulphur plus spreader appeared more variable than that from 10 per cent lime sulphur plus spreader. When disks from leaves sprayed with 3 per cent elemental sulphur were used as a source of sulphur vapor, the mildew inhibition zones averaged 10.5 mm. in diameter after 31 hr. at 24° C., while for 10 per cent lime sulphur they averaged 16 mm. The greater activity of the dried deposit of lime sulphur, as compared with elemental sulphur of similar sulphur content in the original spray, may have been because of the known smaller sulphur particles resulting from the decomposition of lime sulphur, or may have been due to undetermined causes.

A range of lime-sulphur concentrations was tested as a source of sulphur deposit. Naturally the diameter of the inhibition zone increased with the concentration of lime sulphur used. Since studies were conducted over a wide range of temperatures, and the action was slow at the low temperatures, it was felt desirable to use as concentrated a solution as possible in order to get measurable inhibition zones in as short a time as possible. The 10 per cent concentration of lime sulphur was chosen because it was about as high as could be used without injury to the sprayed leaf.

For disks bearing the sulphur deposit, sizes from 3.5 to 21 mm. in diameter were tested. The greater the size of the disk the greater the zone of inhibition beyond the margin of the disk, but the more likely was complete inhibition of mildew development over the entire leaf surface, with the result that the zone of inhibition could not be properly measured. Therefore the small disks were used, though it is realized that the variation in sulphur deposit per disk probably increases with decreasing size of disk.

RESULTS

By use of the methods described, mildew inhibition zones were measured for exposures to sulphur of periods of 10 min. to 100 hr. and at temperatures ranging from 7° to 43° C. As preliminary data were secured, exposure time periods more and more appropriate for each temperature were chosen in successive experiments. For each temperature, the time (on a logarithmic scale) of exposure to sulphur was plotted against the diameter of the inhibition zone, and the results (fig. 2) indicate that this response may be a straight-line relationship. The slopes of these dosage response curves expressed as $\frac{\text{millimeters of inhibition zone}}{\text{logarithmic units of hours of exposure to sulphur}}$ varied from about 12 to 19 and averaged 16, but were probably not significantly different, nor was there any apparent tendency of the slopes to vary progressively with temperature. The data may not be adequate for such a generalization, but the available information indicates that within the range of 4 to 15 mm. diameter for the inhibition zones, each doubling of the time of exposure to sulphur increased the diameter of the zone of inhibition by about 5.5 mm.

In order to compare mildew inhibition by sulphur at different tem-

peratures it was necessary or desirable to choose an arbitrary lethal dose, or in this case an arbitrary diameter of the inhibition zone. A diameter of 1 cm. was chosen, but there is no reason to believe other values might not be as satisfactory. If the dosage response relation is a straight line of constant and determined slope as indicated above, then the time for inhibition of mildew over a 1-cm.-diameter area can be determined from a single observation. This assumption was applied to all data for all temperatures and the results are presented in figure 3. Determinations on this assumption give a great range of values, and from these values it has been calculated that there is an average of 4.9-fold variation in the lethal dose time at any given temperature. Within a given test values were fairly uniform, but between tests variation was great. Some of the causes of this variation may have been such unstandardized factors as dosage of inoculum, age and condition of inoculated leaves and sulphur-treated leaves, variation in lime sulphur, and variation in sulphur deposit. In spite of these variations, however, there is an apparent straight-line relation between temperature and the logarithm of the time of exposure to sulphur for inhibition of mildew in a 1-cm.-diameter zone. Over the temperature range of 7° to 43° C., the temperature coefficient, or relative increase in activity for each 10° C. rise in temperature, was about 4.8. This relation of temperature to the time necessary for inhibition of a 1-cm. area is replotted without data, in comparison with the relation of temperature to the activity of sulphur as reported by other investigators, in figure 4. The writer's data are at variance with those of others.

In tests of the relation of temperature to the fungicidal action of sulphur upon bean rust, detached bean leaves inoculated 3 to 5 days previously with urediospores of *Uromyces phaseoli*, were placed on 40 × 50 mm. glass cylinders within wide-mouth pint jars. In the bottom of each jar were placed 50 cc. of a solution of sodium sulphide in distilled water, and within the jars was placed a crucible containing 2 cc. of 10 per cent sulphuric acid, which, after mixing with the sodium sulphide solution, gave a reaction of pH 1.8. After the jars were sealed and placed at the desired temperatures, the sulphuric acid and sodium sulphide solutions were mixed, with resultant evolution of hydrogen sulphide and perhaps other gases. After 7 hr. at the test temperature the leaves were removed from the jars and held at 24° C. in Petri dishes containing 8 per cent sucrose. Suitable controls were maintained, and after an incubation period of about 10 days from inoculation, the development of rust was noted. With the sodium sulphide dosage increment of 2 which was maintained in these tests, rust development was usually normal, as in the controls without sulphur, or was completely suppressed, giving a very steep dosage response relation.

With such results it would be very difficult to determine a value such as the LD95, and this was not attempted. The critical dosage used was the lowest dosage necessary for complete suppression of rust development. In 5 tests, this averaged 0.00035 per cent sodium sulphide at 37°, 0.0015

per cent at 25°, and 0.02 per cent at 13° C. While these data for bean rust are fragmentary in comparison with those above for bean mildew, both with respect to number of temperatures explored and number of replications, they indicate that the relation of temperature to the fungicidal action of the vapor from sodium sulphide acting as a therapeutant for bean rust may not be significantly different from the results obtained with bean mildew as described above.

DISCUSSION

This paper might be considered a preliminary study of the temperature coefficient of the action of a disinfectant. According to Miller (11), temperature coefficients of physical reactions usually range from 1 to 1.5, while those of chemical reactions usually range from 2 to 3. For biological reactions, temperature coefficients are more variable and may be much higher. For the disinfectant action of various chemicals upon various bacteria the temperature coefficient ranged from 1.5 to 54, according to Rahn (13); for the killing of tobacco mosaic with heat it was about 35 at 75° to 85° C., and about 1000 at 85° to 95° C., according to Price (12); for the killing of bean rust mycelium with heat it was about 44 at 35° to 55° C., according to Yarwood (17); and for the spontaneous destruction of hemolysin it was 71,710 at 20° to 30° C., according to Buchanan and Fulmer (3). It is therefore difficult to relate the temperature coefficient of 4.8 observed in this study to the temperature coefficients of other biological reactions, other than to say that it lies in the lower range of values.

For physical and chemical reactions, temperature coefficients usually decrease with increasing temperature, but biological data in the sources indicated above show examples of temperature coefficients both increasing and decreasing with increasing temperature. With respect to sulphur, the temperature coefficient for the vapor pressure within the range of these trials is, by extrapolation of the data of Menzies (10), about 2.4 and shows a gradual decrease with increasing temperature; while the rate of vaporization according to Liming (6) was about 26 at 25° C. and decreased rapidly to about 4.1 at 45° C. Within the error limits of the writer's data, no change in the temperature coefficient is apparent over the 36° C. range of these tests. A change in the temperature coefficient such as that indicated by Menzies' data could easily be covered up by the variation in the writer's data, but a change in rate such as that indicated by Liming should be apparent. More precise data, which can probably be easily obtained, will be necessary to determine whether or not there is a real change in the temperature coefficient for the fungicidal action of sulphur.

SUMMARY

When leaf disks bearing a dried deposit of lime sulphur were placed on the surface of mildew-inoculated detached bean leaves and later removed, mildew was inhibited for varying distances beyond the position of the

disks, and these distances increased with increasing time of exposure to sulphur from 10 min. to 100 hr. and with increasing temperatures of exposure from 7° to 43° C. The inhibition effect was independent of contact of the sulphur with the inoculated leaf, for when the sulphur-bearing disk was separated from the inoculated leaf by a cheesecloth screen, the distance of inhibition of mildew was similar to that observed when no screen was used. When the diameter of the mildew inhibition zones in millimeters was plotted against the logarithm of the time of exposure to sulphur, the time-response relation was approximately a straight line with a slope of 16. When the logarithm of the time of inhibition of a zone 1 cm. in diameter was plotted against temperature, the temperature-time relation was approximately a straight line with a temperature coefficient of 4.8.

When bean leaves inoculated with rust 3 to 5 days previously were placed in sealed jars with the vapors from sodium sulphide solutions, the rust mycelium was killed without leaf injury if the pH and concentration of the sodium sulphide, and the time and temperature of exposure to sodium sulphide, were properly adjusted. When the logarithm of the concentration of 50 cc. of sodium sulphide at pH 1.8, necessary for complete therapy of bean rust in 7 hr., was plotted against the temperature during exposure to the sulphur vapors, the temperature-concentration relation was approximately the same as the temperature-time relation for the eradication of bean powdery mildew described above, but was not studied so thoroughly.

The temperature coefficient for the fungicidal action of sulphur, as reported in this paper, is compared with other temperature coefficients previously described.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

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BIOLOGICAL RACES OF THE FUSARIUM CAUSING WILT OF COWPEAS AND SOYBEANS[1]

G. M. Armstrong and Joanne K. Armstrong[2]

(Accepted for publication September 1, 1949)

INTRODUCTION

After the discovery that the cotton-wilt *Fusarium* attacks several hosts, namely, cotton, Burley tobacco, okra, and Cassia (1), studies of other wilt Fusaria were made to determine if a lack of specificity occurs more frequently than has been generally assumed. Wilt of cowpea (*Vigna sinensis* Endl.) was included in this investigation since it is rather widely distributed and occurs commonly in South Carolina. Cromwell (4) reported that the Fusarium wilt or blight of soybean (*Soja max* (L.) Piper) is caused by the cowpea-wilt *Fusarium*, hence the soybean was included in the tests, as well as plants of other genera and species. Soybean wilt apparently has a more limited range than cowpea wilt since Johnson and Koehler (6) listed only South Carolina, North Carolina, Louisiana, and Alabama as States from which the former disease has been reported.

Orton (12) planted both soybeans and cowpeas in a field heavily infested with the cowpea-wilt *Fusarium* and noted that only the cowpeas wilted. Cromwell (4, 5) observed that in some localities of North Carolina soybean plants wilted while in others they remained free of disease, although in both instances the soil was infested with the cowpea-wilt fungus. From morphological and cultural studies he concluded that the species of *Fusarium* attacking soybean could not be distinguished from *Fusarium tracheiphilum* Smith by any well defined differences. He stated further: "Since the possibility existed that they might be separated by biological differences, reciprocal inoculation studies were undertaken to secure additional evidence of their identity." These experiments, conducted in the greenhouse and in the field, were not always successful but he concluded that "strains from soybeans and cowpeas showed that cross-inoculations can be made." In view of the finding of biological races which will be described in this paper, it seems probable that Cromwell was confronted with races without recognizing them.

Lehman (10) found that blackeye cowpeas growing in pots of sandy loam soil were more susceptible to Fusaria isolated from wilted cowpeas than to those isolated from wilted soybeans. In comparable tests, however, neither of the strains produced wilt of soybeans.

From the results of greenhouse tests, field experiments, and field obser-

[1] Contribution of the Department of Botany and Bacteriology in cooperation with the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Department of Agriculture. Technical contribution No. 161, South Carolina Agricultural Experiment Station. Acknowledgment is given to C. C. Bennett for aid in certain phases of the greenhouse investigations.

[2] Head, Department of Botany and Bacteriology, and Agent, Division of Cotton and Other Fiber Crops and Diseases, respectively.

vations, Kendrick and Snyder (9) state that *Fusarium oxysporum* f. *tracheiphilum*, which causes cowpea (*Vigna sinensis*) wilt, does not attack soybean (*Soja max*), contrary to statements found in literature (4), nor are common beans (*Phaseolus vulgaris*) or Lima beans (*P. limensis* var. *limenanus*) affected by the cowpea-wilt *Fusarium;* furthermore, "these data show that the bean yellows *Fusarium*, which attacks varieties of *P. vulgaris*, does not affect the varieties of cowpea, soybean, or Lima bean reported upon here. These facts are in line with the recognized tendency of the formae of *F. oxysporum* to be rather narrowly specialized, usually to single genera." All the varieties in these tests are not given but the results herein reported show that this designation is necessary.

Smith (14) was unable to differentiate the Fusaria causing wilt of cotton, watermelon, and cowpeas, respectively, on morphological and cultural characteristics, but he showed by cross inoculations that each *Fusarium* was specific to its host.

MATERIALS AND METHODS

Plants of four varieties of soybeans and 23 varieties or breeding lines of cowpeas have been tested either in field plots or in inoculation experiments in the greenhouse, or in both, for their reaction to numerous isolates of wilt Fusaria from cowpeas and soybeans. In addition, plants of other genera and species have been inoculated with some of the isolates. Enough seed of each variety or breeding line of cowpeas was obtained so that one lot could be used throughout the experiments, since preliminary observations indicated that lots from different sources might show differences in the degree of wilting or in the reaction to the races. Many years ago Piper (13) showed that a variety might have several names, and even the name of another variety. Since several lots of surface-sterilized cowpea seed were found to carry the wilt fungus, a sample of each lot was planted in the greenhouse and only those producing healthy plants were used in the experiments. This difficulty was not encountered with the soybean seed.

In 1947, a plot was selected in a field where a large number of varieties of cowpeas had been grown the previous year and wilt was abundant. Twenty-three lots of cowpea seed representing 17 varieties or breeding lines and the Yelredo variety of soybeans were planted in this plot in randomized blocks with four replications.

In the greenhouse, the sand culture technique, which has been described elsewhere (2), was used. The experiments were conducted chiefly in the fall, winter, and spring when the temperature could be maintained at approximately 28° C. Usually the roots of the plants were cut before inoculation, although in some instances the young soybean plants were removed from the pot, the roots dipped in the inoculum, and the plants reset. The cowpea or soybean seed were surface-sterilized, planted 20 per pot, and the plants inoculated shortly after the first true leaves appeared. With a few exceptions, a plant was removed from a pot as soon as it showed external

symptoms of wilt. If no wilting appeared, the plants were thinned later to 10 per pot. In 6 to 8 weeks, when an experiment was terminated, all the remaining plants were cut diagonally at the base and inspected for vascular discoloration.

Five isolates from wilted soybeans, three from South Carolina and two from North Carolina, and 26 isolates from wilted cowpeas were used in the pathogenicity tests. Three of the cowpea isolates were obtained from non-inoculated plants grown in steamed sand and probably were seed-borne (7). Other isolates were obtained from wilted plants in experimental field plots; from plants collected during a field survey in Georgia, North Carolina, and South Carolina; and from cultures supplied by other workers. A fluffy mycelial single-spore line was derived from each gross isolate and maintained on potato-dextrose-agar slants. After two weeks' incubation at 28° C., the stock cultures from these lines were stored in a refrigerator.

Tests to determine the pathogenicity were made soon after an isolate was obtained. There were concurrent tests in which either a few isolates were tested on a number of cowpea and soybean varieties, or a number of isolates were tested on a few varieties. In most cases the gross isolate was also tested for comparative purposes on the Yelredo soybean and several cowpea varieties. When plants of several varieties were inoculated with an isolate on the same day, a portion of the same inoculum was used for each. Every series included check pots of noninoculated plants of the varieties used, as well as pots of plants of susceptible varieties, inoculated, respectively, with each isolate to verify its virulence. There were also replicate tests with some isolates at different seasons to determine the accuracy of the results.

RESULTS

The isolates of the cowpea- and soybean-wilt *Fusarium* which have been studied differentiated into two distinct biological races and probably others. Eight of the cowpea isolates belonged in race 1 and 18 in race 2. Only two isolates of race 1 as compared to eight of race 2 were procured from the field survey. An isolate of race 1 was also obtained from a wilted soybean in a field. Isolates of both races were obtained from plants produced from infested cowpea seed.

From a limited cultural and microscopical study, no constant or significant differences could be observed between the isolates of the races when they were grown under the same conditions. The variations in cultural and spore characteristics were similar to those described for other wilt Fusaria by numerous workers. All isolates belonged to the section Elegans as described by Wollenweber and Reinking (16), or to *Fusarium oxysporum* as emended by Snyder and Hansen (15).

Greenhouse Tests

Inoculation of cowpeas. The mass of data that has been accumulated during the four years that this study was in progress precludes its presenta-

tion in detail; therefore only the principal results are presented. A summary of the results of the inoculations of cowpeas and soybeans, except the Yelredo variety, is given in table 1. For reasons that will be discussed later, the tests with the Yelredo soybean are reported in greater detail (Fig. 2). The reactions of five varieties of cowpeas to individual isolates of races 1 and 2 are presented in a histogram (Fig. 1). Each shaded block in the histogram represents the percentage of wilted plants in one pot. The black portion of each block denotes the period of wilting in days, that is, the interval during which plants showing external symptoms of wilt were removed from a pot. The base line in this case represents the day of the first inoculation. If wilting was noted on one day only, this is shown as a dash. The histogram does not, however, show the severity of either the external or internal wilt symptoms. The plants of varieties such as Lady Finger, Sumptuous, and others were usually killed quickly by the isolates to which they were susceptible, while affected plants of the wilt-resistant Brabham variety usually survived until maturity, even though they had symptoms of wilt. Orton (12) has described the symptoms of wilt of cowpeas and Cromwell (4), those of soybeans.

TABLE 1.—*Results of greenhouse inoculations of cowpeas and soybeans with various isolates of the cowpea-soybean wilt Fusarium*

Varieties	Fusarium isolates							
	Race 1			Race 2			Isolates 30 and 31	
	No. of isolates	No. of plants	Percentage wilt	No. of isolates	No. of plants	Percentage wilt	No. of plants	Percentage wilt
Cowpeas								
Lady Finger	9	152	96.1	18	181	0.0	33	0.0
Florida Conch	4	74	90.5	4	62	9.7	10	0.0
Brown Sugar Crowder	9	188	81.4	18	210	68.1	57	0.0
Calif. Blackeye-5	3	30	0.0	11	134	100.0		
Ramshorn	1	10	0.0	1	10	100.0		
Chinese Red	4	75	4.0	10	140	99.2	20	0.0
Sumptuous	9	92	2.2	18	268	95.5	20	0.0
Dixie Queen	3	34	11.8	2	26	92.3		
Whippoorwill	3	26	0.0	2	25	64.0		
Calva-7	3	30	26.7	11	126	57.1		
Clay	3	40	0.0	3	33	51.5		
Brabham	3	33	18.2	10	101	3.0		
Iron	1	10	0.0	2	20	0.0		
Lady (Morse)	1	23	13.1	1	26	38.5		
Rice (do)	1	10	0.0	1	20	90.0		
Rice or Lady (Andeo)	1	10	0.0	1	11	63.6		
Rice or Lady (Wood)	1	21	4.8	1	26	42.3		
Soybeans								
L. Z.	2	26	0.0	3	34	3.0	23	0.0
Yelnando	4	40	0.0	11	110	0.0	18	11.1
Mammoth Yellow	3	52	1.9	1	10	0.0		

The results presented in table 1 and figures 1 and 2 show that there are definitely two biological races of *Fusarium oxysporum* f. *tracheiphilum* Snyder and Hansen as shown by their selective pathogenicities for certain varieties of cowpeas and soybeans. There are indications that other races probably exist.

Lady Finger and Sumptuous are excellent varieties of cowpeas for differentiating races 1 and 2 (Fig. 1). The former is highly susceptible to iso-

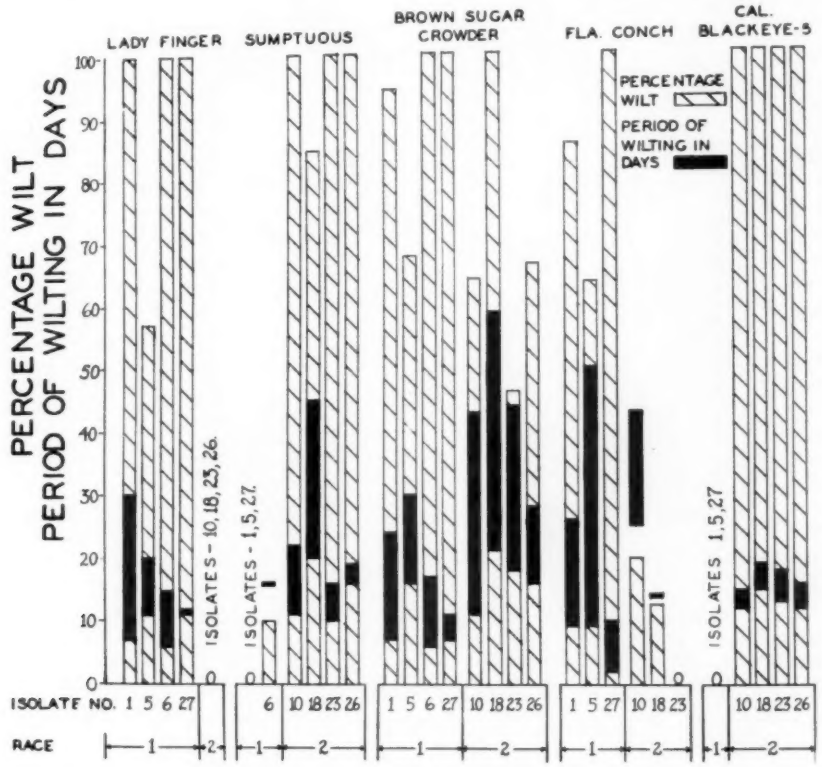

FIG. 1. Percentage wilt and period of wilting, in days, for five varieties of cowpeas inoculated with isolates of races 1 and 2 of the soybean-cowpea-wilt Fusarium. Each shaded bar of the histogram represents the results obtained with one pot of plants. Period of wilting is the time interval, measured from day of first inoculation (represented by base line), during which plants were removed from the pots as they showed the first external symptoms of wilt.

lates of race 1 and very resistant to those of race 2, while the latter shows the converse reaction. California Blackeye-5 could also be used since it showed the same reaction as Sumptuous. The root-knot-nematode-resistant Florida Conch reacted to the two races approximately the same as Lady Finger, the difference being its slight susceptibility to race 2 (Fig. 1). Other lots of seed labeled Lady[3], Rice or Lady, or Rice[3] produced plants

[3] These lots kindly supplied by W. J. Morse of the U. S. Department of Agriculture.

resistant to race 1 and susceptible to race 2 (Table 1), which indicates that this lot of Lady Finger[4] seed is not the same as Lady. Other varieties which were susceptible to race 2 and resistant to race 1 are given in table 1. The Brown Sugar Crowder variety (Fig. 1) was susceptible to both races, although it was not so susceptible to race 1 as Lady Finger nor to race 2 as some varieties.

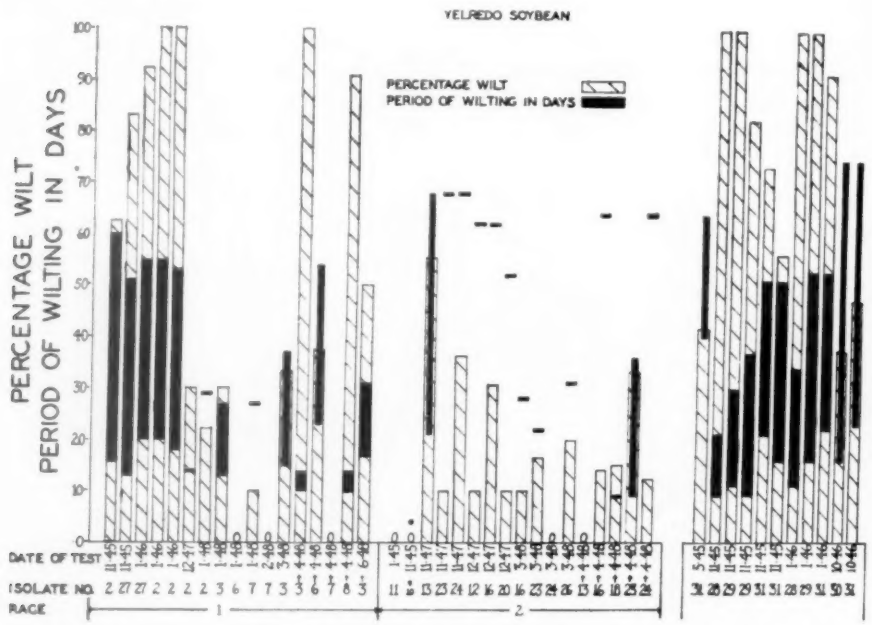

FIG. 2. Percentage wilt and period of wilting, in days, for Yelredo soybean inoculated with isolates of races 1 and 2 of the soybean-cowpea-wilt Fusarium, and other isolates from soybean. Each shaded bar of the histogram represents the results obtained with one pot of plants. Period of wilting is the time interval, measured from day of first inoculation (represented by base line), during which plants were removed from the pots as they showed the first external symptoms of wilt. * Each isolate used separately. † Roots dipped in inoculum.

Of all the varieties, Iron and Brabham were the most resistant to both races. The reaction of Iron to an isolate of each race indicated that it is more resistant than Brabham since there were no external and, in a few plants, only slight internal symptoms of wilt. Most of the plants of Brabham inoculated with isolates of race 1 showed marked discoloration of the stele, extending halfway or more up the stem. As these plants approached maturity, a few of the basal leaves turned a uniform yellow and were shed. Since the shedding of yellowed lower leaves occasionally occurred on non-inoculated plants, this could not be used as a definite criterion of wilt for Brabham, nor in some instances for the semiresistant Calva-7 (8). The slightly greater leaf shedding of the inoculated plants of the resistant varieties was ascribed to the effects of the wilt fungus only after noting the vascular discoloration of the stem. The shedding of the lower leaves by

[4] Piper (13) reports that seed received by him as Lady Finger proved identical with Lady.

Brabham and the reaction of two other varieties when inoculated with an isolate of race 1 are shown in figure 3. All plants of Lady Finger were killed, whereas those of California Blackeye-5 were vigorous and showed no yellowing or shedding of the lower leaves.

FIG. 3. Reaction of three varieties of cowpeas to race 1 of the cowpea-wilt Fusarium. Left, Brabham (shedding of a few lower leaves). Center, Lady Finger. Right, California Blackeye-5.

The following comparisons were possible since fresh isolates were used in most of the pathogenicity tests and replications of the tests performed at different times generally gave comparable results.

Where wilting occurred, the symptoms were similar after inoculation with either race 1 or 2, but usually appeared sooner with race 1 than with race 2. This was evident in the differential varieties and also in Crowder which was susceptible to both races.

The isolates of a race varied somewhat in the degree of virulence. This was evident in the time required for the appearance of the first symptoms of wilt, the rapidity of killing of plants, and the percentage of wilted plants.

Some striking differences in the discoloration of the stele were evident among the varieties. Lady Finger and Sumptuous had very little discoloration with the race to which each is resistant, perhaps only a slight streak in the basal region of the stem in a few plants. This is another reason why they serve as excellent differentials to separate races 1 and 2. On the basis of external symptoms of wilt, Florida Conch, susceptible to race 1, and Chinese Red, California Blackeye-5, Dixie Queen, Whippoorwill, and Clay, all susceptible to race 2, also served as differential varieties. Considerable vascular discoloration occurred, however, in a high percentage of the plants inoculated with the race to which each is otherwise resistant.

Inoculations of soybeans. The Yelredo soybean is generally susceptible to isolates of race 1, whether obtained from cowpeas or soybeans (Fig. 2.). Only one isolate of this race, isolate 27, was obtained from soybeans but it was as virulent on the varieties of cowpeas susceptible to the race as any of the isolates obtained from cowpeas. This variety is considered generally resistant to race 2 since the symptoms were usually slight if wilt appeared. Some exceptions are indicated, as in one test with isolate 13 where 55 per cent wilt occurred. Indications of a third race are given by isolates 28 and 29 which were obtained from wilting soybeans in North Carolina[5]. In early tests, these isolates were virulent for Yelredo soybeans but later lost pathogenicity. Before the loss of pathogenicity for soybeans, the isolates were used to inoculate the Brown Sugar Crowder cowpea, but unfortunately this lot of seed carried the wilt fungus, since there were a few wilting plants in the noninoculated pots. However, there were fewer wilting plants in the inoculated series. This indicated that the two isolates probably belong to a race which was pathogenic to Yelredo soybeans but not to Brown Sugar Crowder cowpeas, in contrast to isolate 27 from soybeans which caused wilt of certain varieties of both cowpeas and soybeans. An analysis of the results of Cromwell (4) suggests that this is probably the correct interpretation. Another culture[5] isolated later from a soybean plant collected in the same locality of North Carolina proved to be so weakly pathogenic for Yelredo soybean that the true situation remains uncertain.

The two soybean-wilt isolates from South Carolina, numbers 30 and 31, caused wilt of Yelredo soybeans but not of cowpeas (Fig. 2 and Table 1). When these were tested along with isolates 28 and 29 on hosts other than soybeans or cowpeas they were found to have different host relationships. Therefore, it seemed logical to put them with another race in a host complex to be given later.

Plants of the Yelnando, L. Z., and Mammoth Yellow varieties of soybeans were inoculated in separate pots with isolates which were representative of races 1 and 2 and the other probable races. With the Mammoth Yellow

[5] Courtesy of Dr. S. G. Lehman.

variety, some inoculations were made by the usual method of cutting the roots of plants growing in the pots, and others by dipping the roots of young plants in the inoculum and then setting the plants in pots. These varieties were wilt-resistant, since only a few plants had external symptoms of wilt. Furthermore, only a few additional plants had a slight discoloration of the vascular tissue in the basal region of the stems. These results show the necessity of specifying varieties used in an investigation of the fusarium-wilt diseases.

Inoculations of other plants. Plants of the genera, species, or varieties listed below were grown in pots of sand and inoculated with each of one or more isolates of race 1 and race 2, respectively. There were five plants of tobacco or sweet potato, and ten or more of all other plants per pot. No external symptoms of wilt appeared on any of the plants inoculated with a heavy mass of inoculum, which indicates that they are not wilt-susceptible hosts of these races. Vascular discoloration was present in such few cases, with most plants, that it is probably insignificant. However, an appreciable number of plants of cassia, alfalfa, and pigeon pea showed slight internal discoloration with race 1.

Alfalfa (*Medicago sativa* L.) varieties Grimm and Kansas Common
Cabbage (*Brassica oleracea* var. *capitata* L.) var. Copenhagen Market
Coffeeweed (*Cassia tora* L.)
Sea Island cotton (*Gossypium barbadense* L.) var. Seabrook
Upland cotton (*Gossypium hirsutum* L.) varieties Rowden and Coker 100 Wilt.
Crotalaria juncea L.
C. mucronata Desv. var. Giant Striata
C. spectabilis Roth
Mimosa (*Albizzia julibrissin* Duraz.)
Okra (*Hibiscus esculentus* L.) var. Clemson Spineless
Garden pea (*Pisum sativum* L.) varieties Alaska Extra Early and Thos. Laxton
Pigeon pea (*Cajanus cajan* (L.) Millsp.) var. White Pea, early
Sweet potato (*Ipomoea batatas* (L.) Lam.) var. Porto Rico.
Sesame (*Sesamum indicum* L.) varieties Ajonjoli Criollo and P.I. 153508
Snapdragon (*Antirrhinum majus* L.) var. Loveliness
Spinach (*Spinacia oleracea* L.) var. Bloomsdale Savoy Leaved
Tobacco (*Nicotiana tabacum* L.) varieties Burley 5 and Gold Dollar
Hairy vetch (*Vicia villosa* Roth)

Cowpeas of at least two varieties which were differentials for races 1 and 2 were inoculated with virulent fusarium-wilt isolates from cabbage, coffeeweed, cotton, crotalaria, mimosa, okra, garden pea (race 2), sweet potato, sesame, spinach, and both Burley and Gold Dollar tobacco. There were no external symptoms of wilt in any case.

Field Tests

Early in the season, counts of plants with fusarium wilt could be made with certainty, but later, complicating factors developed to affect their accuracy. Many plants showed the presence of either *Macrophominia phaseoli* (Maubl.) Ashby, *Rhizoctonia solani* Kuehn, *Sclerotium rolfsii* Sacc., or the lesser corn stalk borer. For this reason the results in the field are not reported in detail.

There were approximately 300 plants of each variety in the plot. The number of plants of a few varieties removed from the plot between June 17

and August 15, all of which were dead or nearly dead from wilt, were: Brabham, 1; Calva-7, 3; Lady Finger, 6; Florida Conch, 16; California Blackeye-5, 79; Chinese Red, 157; and Sumptuous, 215. The variable reaction of a variety when the seed was obtained from three sources, is shown by Brown Sugar Crowder with 194, 128, and 35 dead plants; and from two sources, by Dixie Queen with 293 and 83 dead plants.

Two races of the cowpea-wilt *Fusarium* were obtained in 1946 from wilted plants in the field plot, but it appeared in 1947 that race 2 greatly predominated since some of the cowpea varieties very susceptible to this race were completely eliminated in two of the four blocks, whereas the varieties susceptible only to race 1 had a small percentage of wilt. No wilt was observed in the Yelredo soybeans.

DISCUSSION

Portions of a lot of infested cowpea seed were first sown in steamed sand and in a garden the two following years. Isolates of race 1 were secured from wilting plants in each case. When seed of this lot, which had been kept in a refrigerator for 4 years, were again sown in steamed sand, some of the plants wilted and a *Fusarium* belonging to the same race again was isolated. In both tests the seed were treated with sodium hypochlorite before planting in steamed sand. Thus, one is led to infer that the fungus may be carried in the seed since this treatment has been shown (2) to free the surface of wooden sticks of the wilt Fusaria. Kendrick (7), however, has implied that the fungus is chiefly on the surface of the seed.

It appears from the field survey that race 2 occurred more frequently than race 1; but this may not be true, since it is probable that the varieties most commonly grown in the area were those attacked by race 2. If antagonism between the races exists, as has been shown for races of flax wilt *Fusarium oxysporum* Schlecht f. *lini* (Bolley) S. and H. by Borlaug (3), this may also be a factor in the relative prevalence of the races.

A comparison of the results of this investigation with those of Kendrick and Snyder (9) was presumably possible by means of isolate 12 which was obtained from an internally discolored plant in a pot of noninoculated plants of California Blackeye-5[6]. A comparison of this isolate with isolate 26[6], which was received later, showed that both belonged to race 2. One would infer, therefore, that they were confronted with the fungus of this race and, if they used a wilt-susceptible variety of soybeans, no wilting of the latter would be expected on the basis of our results. Probably this would also explain their disagreement with the results obtained by Cromwell. Orton also observed no wilting of soybeans in soil infested with the cowpea-wilt *Fusarium*, which indicates that race 2 may have been the one present.

Mackie (11) developed California Blackeye-5 as a wilt-resistant variety. Since it is very resistant to race 1 but susceptible to race 2, the question arises whether Mackie's breeding plots were infested with race 1 while those of Kendrick were infested with race 2.

[6] Seed and culture kindly supplied by Dr. J. B. Kendrick.

In conducting wilt tests with Fusaria an ever-present problem is the loss of pathogenicity by some isolates. For this reason, cultures should be tested soon after isolation so that their full potentialities can be determined. Tests with cowpeas could be repeated with a degree of certainty but this was not always true with soybeans. From the soybean varieties used, and the situation noted in the field, the impression was obtained that perhaps most varieties of soybeans are wilt-resistant. Upon finding the Yelredo[7] variety wilt-susceptible, most of the experiments were performed with this variety. Yet with this susceptible variety there were some unexplained variations. For example, in an inoculation experiment with an isolate initiated from a single spore, all of the plants wilted. An inoculation about 2 weeks later with inoculum derived from another culture of the same isolate gave only 40 per cent wilt. In some cases, a high percentage of wilt in an experiment was followed later by no wilt. If the isolate had lost its pathogenicity for soybeans, concurrent and later tests indicated that it retained its virulence for cowpeas. Most of the isolates have retained their virulence for cowpeas, although some have been kept in culture for five years.

In several instances in which an isolate caused wilt of all soybean plants in a pot and of only a few in a subsequent test, single-spore cultures of the *Fusarium* were made from the latter. These also caused wilt of only a small percentage of the plants, and like the original virulent isolate, quickly produced wilt symptoms, but only in a few plants. Nearly all the remaining plants failed to show even a slight internal discoloration. A thorough study of the factors involved in the wilt of soybeans is needed.

Systems of Classification of Fusaria

The present paper is limited to a presentation of the data on the pathogenicity of the Fusarium isolates. However, the system of classification by which the fungus will be named must be considered. Tentatively, the name *Fusarium oxysporum* f. *tracheiphilum* suggested by Snyder and Hansen (15) is used; however, evidence suggested in this paper (some soybean-wilt isolates) and facts to be presented in later papers will show the need for modifications of this system. These will be suggested in later papers.

SUMMARY

Observations of a few varieties of soybeans and numerous varieties or breeding lines of cowpeas in a field plot, as well as inoculation of these in the greenhouse with fusarium-wilt isolates from both hosts, indicated that there are definitely two and probably other biological races on the basis of pathogenicity.

Race 1 obtained from both soybeans and cowpeas caused wilting of some varieties of both hosts; while race 2, obtained only from cowpeas, caused severe wilting only of some varieties of this host.

[7] In a personal communication from Dr. H. W. Johnson this variety was suggested since it had the most wilt in field plots.

The cowpea varieties, Lady Finger and Sumptuous, served as excellent differentials for races 1 and 2 since they were killed rather quickly by the race to which each was susceptible but showed practically no symptoms of wilt, either external or internal, with the race to which each was resistant. The Brown Sugar Crowder variety was susceptible and the Iron and Brabham varieties were generally resistant to both races 1 and 2. Some varieties showed few external symptoms of wilt after inoculation, but vascular discoloration was rather extensive.

Indications were obtained of a race from soybeans which caused wilt only of this host, but loss of pathogenicity of the isolates made it impossible to prove the point.

Another group of isolates from soybeans which caused wilt of soybeans but not of cowpeas has different host relationships and will be discussed later in another host complex.

Some isolates lost pathogenicity for soybeans but not for cowpeas.

No significant cultural or microscopic differences could be detected between the races, although the isolates represented a wide range of cultural types.

Several lots of cowpea seed carried the wilt *Fusarium* which was not eliminated by seed treatment with a sodium hypochlorite solution.

Observations of many varieties of soybeans in the field and the inoculation of plants of a few varieties in the greenhouse indicated that most varieties may be wilt-resistant.

Pathogenicity tests with cowpeas were repeated with comparable results. Some unexplained variations occurred in the tests with soybeans, indicating that further work is needed on the factors involved in the wilting of soybeans.

Inoculation experiments with one or more isolates of both races 1 and 2 on certain varieties of the following plants failed to produce external symptoms of wilt: alfalfa, cabbage, coffeeweed, cotton, crotalaria, mimosa, okra, garden pea, pigeon pea, sweet potato, sesame, snapdragon, spinach, tobacco, and hairy vetch. There were also no external symptoms of wilt when cowpeas were inoculated with the wilt Fusaria from most of these hosts.

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CLEMSON, SOUTH CAROLINA

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OCCURRENCE OF STRAINS OF THE STRAWBERRY RED STELE FUNGUS, PHYTOPHTHORA FRAGARIAE HICKMAN, AS SHOWN BY DIFFERENTIAL VARIETAL RESPONSE

D. H. Scott, W. F. Jeffers, George M. Darrow, and D. P. Ink[1]

(Accepted for publication September 6, 1949)

When the red stele disease first became known in the United States (1, 7), strawberry varieties were tested for resistance to it and Aberdeen was the only American variety found to be highly resistant. Breeding work for red stele resistance was initiated using Aberdeen as a parent, and from that work the resistant varieties Temple, Pathfinder, Sparkle, and Fairland were obtained (4, 5, 8). These varieties are now in commercial production in the Northeastern States.

In 1938 several strawberry selections that had originated in Scotland, where the red stele disease had been serious for a number of years, were tested for red stele resistance in Maryland. Some of these selections were highly resistant and accordingly they were used as parents in the breeding program.

Preliminary observations in the early phases of the breeding work for resistance indicated that some strawberry varieties and selections that were resistant in Maryland were susceptible when grown in other States. Since it appeared that there might be strains of the causal organism, a resistance test plot was established in which red-stele-infested soils from a number of different fields were spread on the land and disked in (9, 10). When planted in this soil in both field and greenhouse bench tests, Aberdeen and all seedlings from it became severely infected with the red stele fungus. However, in these same plots Md. 683 (Scotland BK46 × Fairfax) and US-3374 (Dorsett × Scotland CC18) were highly resistant and transmitted high resistance to some of their seedling offspring when crossed with susceptible varieties (6). Thus it appears that either the Scottish type of resistance is of more value in breeding than resistance derived from Aberdeen, or there are different strains of the organism.

The question whether there are strains of the red stele organism has a direct bearing on the breeding for disease resistance. Since the organism is very difficult to isolate (2, 3), it has not been possible thus far to obtain pure cultures for determination of strains. Therefore, it was decided to grow a number of strawberry varieties and selections in red-stele-infested

[1] Geneticist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture; associate plant pathologist, Maryland Agricultural Experiment Station; principal horticulturist and scientific aide, respectively, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

TABLE 1.—*Reaction of 10 varieties and selections of strawberries to red stele disease obtained from different soils, expressed as percentage of plants occurring in the different classes of infection*

Variety	Soil[a] class of infection, and percentage of plants in each															
	Soil B				Soil C				Soil D				Soil E			
	Severe	Moderate	Slight	None	Severe	Moderate	Slight	None	Severe	Moderate	Slight	None	Severe	Moderate	Slight	None
Aberdeen	0	0	21	79	64	28	8	0	96	4	0	0	0	0	8	92
Fairland	0	0	8	92	100	0	0	0	92	8	0	0	0	0	4	96
Mastodon	0	4	17	79	48	28	20	4	76	12	8	4	54	29	17	0
Ore. 1985	0	0	0	100	72	20	8	0								
Pathfinder	61	26	4	9	76	16	8	0	92	0	8	0	0	0	20	80
Sparkle	55	40	0	5	72	28	0	0	50	33	17	0	0	0	13	87
Temple	21	0	21	58	96	4	0	0	88	8	4	0	0	0	4	96
Md. 683	0	0	29	71	0	0	38	62	0	4	80	16	84	16	0	0
Md. 1972[b]	0	0	29	71	0	0	24	76	0	0	76	24	52	16	32	0
Blakemore	44	36	0	20	88	12	0	0	100	0	0	0	100	0	0	0

[a] In a control lot in Soil A, 100 per cent of plants of all varieties were free of red stele.

[b] Md. 1972 = Blakemore × Md. 683.

soils obtained from different sources to see whether there would be differential responses.

METHODS AND MATERIALS

The work was done in a greenhouse at Beltsville, Maryland, in the winter of 1948–1949. A raised, water-tight greenhouse bench was divided into five sections, each 3 × 4 ft., 8 in. deep. A space of 1 ft. was left between each section to prevent contamination. Each section was filled with one of the five different soils. Soil A was regular potting soil which had been steamed. Soil B was steamed potting soil inoculated with red-stele-infected plants from Oregon.[2] Soil C was red-stele-infested bench soil which had been used the two previous years at Beltsville in testing for red stele resistance. It originally came from a test plot on the Maryland Eastern Shore. Soil D was obtained directly from an area in the same test plot from which soil C was taken. Soil E was taken from a commercial strawberry field on the Maryland Eastern Shore where the Temple variety had not been infected, but where Howard 17 had been killed by red stele.

The test varieties and selections used are listed in table 1. Blakemore is highly susceptible to red stele. All others had been resistant under some of the conditions where tested.

The plants were set on November 20, 1948, with a spacing of 1½ in. in the row and 3 in. between rows. There were five plants of each variety per plot and five replications of plots in each soil. Every third row was planted with Blakemore, a highly susceptible variety, in order to determine uniformity of infection and to aid in interpreting results.

RESULTS

The plants were examined for red stele infection on March 1, 1949, and the results are in table 1. Plants were classified according to degree of infection depending on how severely the root systems had been affected by the disease. Severely infected plants were those in which all or nearly all of the root system was dead. Plants moderately infected had some roots with part of the stele undamaged and some with the stele dead to the crown of the plant. Slightly infected plants had a trace of infection at the tip on only an occasional root. Some plants showed no visible evidence of infection and were classed as clean.

The critical reaction point for the plants tested is between those moderately infected and those slightly infected. Usually any variety or selection that is severely or moderately infected is too susceptible to be of commercial value in red-stele-infested soil, whereas any plants in the other two classes are practically uninjured.

None of the plants grown in the check (soil A) had any infection. The results with the soil B were rather inconclusive as there apparently was not sufficient inoculum to give a thorough test, as is indicated by the 20 percent

[2] Acknowledgment is made to George M. Waldo for the material from Oregon.

of the plants of Blakemore that were free of infection. It is to be noted that Pathfinder and Sparkle were susceptible, whereas most varieties were resistant.

The varieties responded almost exactly the same in soils C and D. Here plants of varieties with resistance derived from Aberdeen (Fairland, Pathfinder, Sparkle, and Temple) were susceptible, while those with resistance from the Scottish parents (Md. 683 and Md. 1972) were highly resistant. In soil E there was a complete reversal of varietal response to red stele in comparison with soils C and D. Aberdeen, Fairland, Pathfinder, Sparkle, and Temple were highly resistant or clean; whereas Md. 683 and Md. 1972 were susceptible. Eight plants of Md. 1972 were classed as having a trace of infection; these were small plants that may not have made sufficient root growth to become severely infected. These last two selections were almost as badly damaged in soil E as Blakemore, which was completely susceptible. Thus, it is evident from the varietal differences in resistance in the different soils (Table 1) that there are at least two strains of the organism causing the red stele disease of strawberries. It is suggested that the strains be designated A and S to indicate Aberdeen type of resistance to the one and Scottish type of resistance to the other.

This differential response of strawberries to strains of the red stele organism is of considerable significance. There is the question of whether other strains exist and, if so, how numerous they may be and how widespread. Breeding for resistance to red stele in strawberries appears more involved than was originally believed to be the case, since at present no variety is known to be resistant to both of the strains now recognized. At Beltsville, three progenies, both parents of which were resistant to one of the two strains, were tested in type C soil carrying strain A. Approximately 1500 seedlings were saved that were resistant to this strain; and it is expected that some of these will also be resistant to strains occurring in type E soil, in view of previous experience (9). To test their resistance to the second strain of the fungus will require another season's work, which doubles the length of time needed to evaluate this material for resistance.

PLANT INDUSTRY STATION
BELTSVILLE, MARYLAND
AND
MARYLAND AGRICULTURAL EXPERIMENT STATION

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CANE AND LEAF RUST, KUEHNEOLA UREDINIS (LINK) ARTH., OF BLACKBERRIES IN WESTERN WASHINGTON

GEORGE W. FISCHER AND FOLKE JOHNSON[1]

(Accepted for publication September 6, 1949)

INTRODUCTION

During the past several years there has been under observation in western Washington a cane and leaf rust of cultivated cutleaf Evergreen blackberry, *Rubus laciniatus* Willd., which has been identified as *Kuehneola uredinis* (Link) Arth. Identification was made possible when the characteristic buff-colored telia were discovered during the late summer and early fall in the vicinity of Puyallup. Apparently this rust has not been reported in the western states, except "sparingly along the coast of southern California" (Arthur). Arthur (1) gives the range otherwise as Nova Scotia to Wisconsin and southward to Florida and Louisiana. Weiss (6) records *K. uredinis* on various combinations of *Rubus* spp. (blackberries, dewberries, and raspberries) in much the same range, including Kansas and Texas. It would appear, then, that the present report would give considerable extension to the geographic distribution of *K. uredinis* in the United States. This is of additional interest in view of its epiphytotic development in western Washington during the past season.

DISTRIBUTION AND HOST RANGE

In Washington blackberry cane and leaf rust was first observed[2] several years ago in the Puget Sound area of Pierce County, but it was not until 1947 and 1948 that it became widespread and severe and a definite factor in commercial blackberry production. In surveying the distribution of the rust during the 1948 fall season, it was found on the cutleaf Evergreen blackberry in every county in western Washington in a direct line from Sumas on the Canadian border to Vancouver at the southern limit of the State (Fig. 1). There is a good reason to suspect, therefore, that the rust occurs also in British Columbia and Oregon. The rust has also been found on the San Juan Islands in recent years.

Apparently there are two forms of the Evergreen blackberry, *Rubus laciniatus*, in the Pacific Northwest (5): the cutleaf, which is the one grown commercially, and the broadleaf. Two strains of the cutleaf form, thorny

[1] Chairman, Division of Plant Pathology, Washington Agricultural Experiment Stations, Pullman, Washington; and Associate Plant Pathologist, Western Washington Experiment Station, Puyallup, Washington, respectively. Published as Scientific Paper No. 833, Washington Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington.

[2] The rust was observed on blackberries in Pierce County in 1940 by Mr. W. W. Baker, who is at present County Agricultural Agent of San Juan County. We are indebted to him for recently sending us samples of infected blackberry leaves from the San Juan Islands.

and thornless, are by far the principal hosts of *Kuehneola uredinis* in western Washington. The rust was found only once on the broadleaf form. In addition, the Pacific Coast dewberry, *R. macropetalus* Dougl., appears to be very susceptible. It was not uncommon to find leaf infections (Fig. 2, A) on only certain vines of this host, while entangled among them would be others that were entirely rust-free. Whether or not these were different species on the basis of Brown's work (2) could not be verified. There appeared to be a slight difference in the morphology of the leaves between the infected and uninfected plants. No rust was found on the western wild black raspberry, *R. leucodermis* Dougl., or on the cultivated Himalaya blackberry, *R. thyrsanthus* Focke.

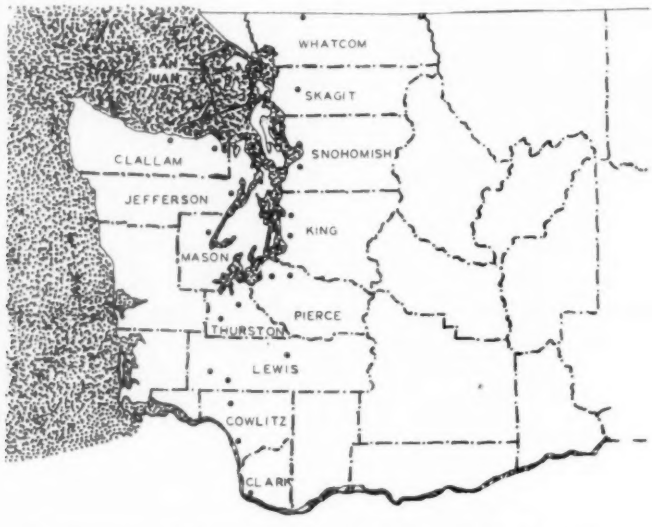

FIG. 1. Distribution of *Kuehneola uredinis* in western Washington. The black dots show where the rust was collected in significant quantities in the counties indicated.

A planting of cultivated blackberry and dewberry varieties at the Southwestern Washington Experiment Station, Vancouver, Washington, and one located at the Western Washington Experiment Station, Puyallup, containing heavily rusted plants of the Evergreen cutleaf blackberry, provided opportunity to observe specific and varietal reaction to the rust. The data obtained are summarized in table 1.

OBSERVATIONS ON LIFE HISTORY

Under western Washington conditions blackberry cane rust makes its first seasonal appearance in the late spring and early summer as large, lemon-yellow uredia splitting open the bark of the overwintered canes (Fig. 2, E). Inasmuch as these are the fruiting canes, the importance of these early lesions is obvious. As the summer progresses, minute yellow uredia appear on the lower surface of the leaves of these fruiting canes. The sub-

sequent build-up of these infections apparently depends upon proper conditions of temperature and humidity during the growing season. In 1948, an unusually wet season, the rust developed on the leaves to the extent of premature defoliation in some plantings of the cutleaf Evergreen black-

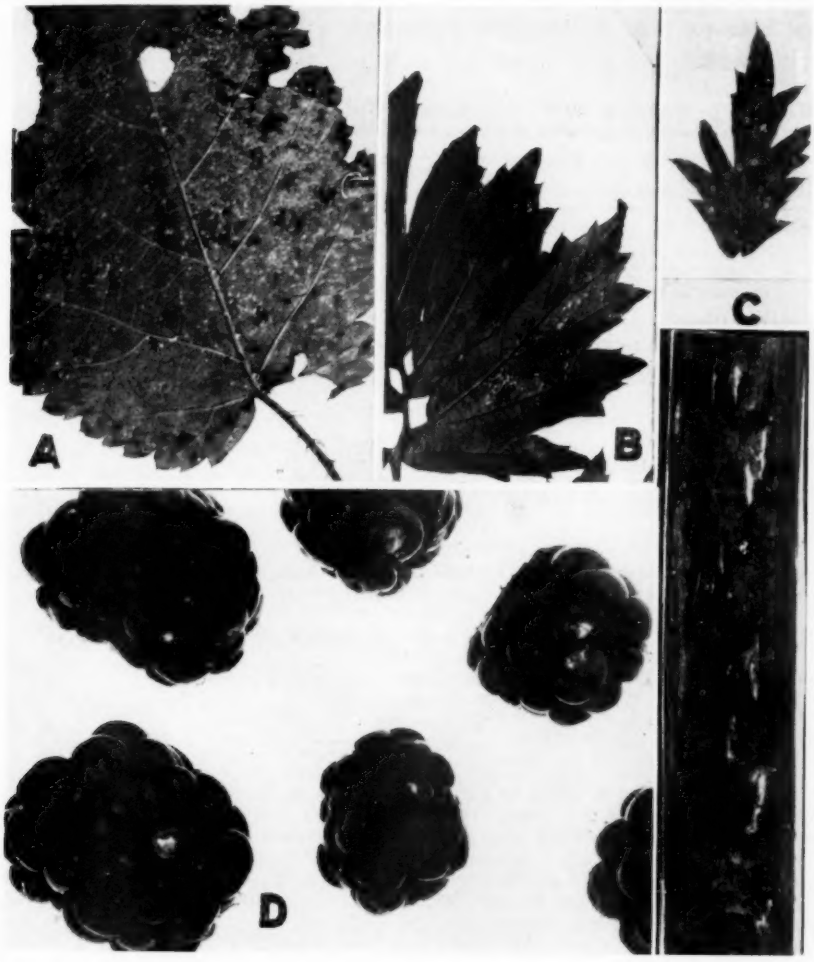

FIG. 2. *Kuehneola uredinis* in western Washington. A. Uredia (hypophyllous) on leaflet of Pacific Coast dewberry. B. Telia (hypophyllous) on cutleaf Evergreen blackberry. (The intermixed uredia are not visible because of the filter used.) C. Aecia (epiphyllous), on leaflet of cutleaf Evergreen blackberry. D. Uredia on ripe fruit of cutleaf Evergreen blackberry. E. Spring uredia on over-wintered canes of cutleaf Evergreen blackberry. All approximately natural size.

berry. In 1948 uredia were observed also on the fruit (Fig. 2, D). While losses due to dockage in the packing sheds were slight in 1948, this phase of the rust has considerable potential importance in this respect. Fruit infections in several fields were severe enough to present a picking problem to avoid including the infected berries.

Telia were not observed until late in September, 1948. These sori were, in most cases, sparingly and rather inconspicuously produced. They appeared as buff-colored areas among the uredia (Fig. 2, B). Whereas uredospores germinated readily in hanging drops of tap water, only one or two isolated instances of germination of teliospores have been observed. This would indicate that the teliospores germinate during the same season they are produced.

TABLE 1.—*Comparative reaction of cultivated blackberries, dewberries, and hybrids to cane and leaf rust, Kuehneola uredinis, in western Washington*

Reaction, and stock[a]	Parentage[b]
Susceptible	
Chehalem	Santiam[c] × Himalaya
Thorny Evergreen	Unknown
Thornless Evergreen	Sport of thorny parent
Intermediate	
Broadleaf Evergreen	Unknown, but believed to be Evergreen × Himalaya
Ore. No. 743	Pacific × Boysen
W.S.C. No. 1	Himalaya × Santiam[c]
Uninfected	
Boysen, Thorny	Unknown
Boysen, Thornless	Sport of thorny parent
Brainard	Himalaya × Unknown
Cascade	Zielinski[c] × Logan
Eldorado	Unknown
Himalaya	Unknown
Logan, Thorny	Aughinbaugh[c] seedling × Antwerp red raspberry
Logan, Thornless	Sport of thorny parent
Lucretia	Unknown
Nectar	Youngberry seedling
Ore. No. 583	Santiam[c] × Young
Ore. No. 616	Zielinski[c] × Logan
Pacific	Zielinski[c] × Logan
Young, Thorny	Phenomenal × Mayes
Young, Thornless	Sport of thorny parent

[a] This classification is based on the usual criteria of the number and size of uredia. "Uninfected" is used rather than "resistant," since these are but one year's observations in the field, no inoculation attempts having been made.

[b] The information on the numbered hybrids was obtained by private communication with G. F. Waldo and C. D. Schwartze, Horticulturists, U. S. Dept. Agr., Oregon State College, Corvallis, Oregon, and Western Washington Experiment Station, Puyallup, Washington, respectively. Other information from Darrow and Waldo (see reference 3, Literature Cited).

[c] Selection from the wild dewberry, *Rubus macropetalus*.

More conclusive evidence of late summer and early fall germination of the teliospores of *Kuehneola uredinis* came with the discovery of pycnia and aecia in late October and early November, 1948 (Fig. 2, C). These sori were most abundant on leaves of those new canes of the current season that were close to the ground and beneath the old fruiting canes. In every such case old telia were found in comparative abundance on the old leaves superimposed over the younger aecia-bearing leaves of the current season.

Thus, all stages of the life cycle of *Kuehneola uredinis* have now been observed in western Washington. How the rust overwinters in this area

has not been determined. Inasmuch as the first appearance of the rust in the late spring is in the form of large uredia on the overwintered canes, it seems probable that it overwinters as latent uredia or as uredial mycelium in the canes. These overwintering infections could come from leaf-borne uredia or from the aecia as observed above. Thus the uredial stage may prove to be sufficient for the perpetuation of *K. uredinis* under western Washington conditions, and the telia and other stages relatively unimportant.

Dodge and Wilcox (4) describe the "late stage" of blackberry cane rust as "often snow white," referring, presumably, to the telia. We also have encountered white sori in late-season infections. However, instead of being telia, which really are buff-colored, these white sori appear to be parasitized uredia. They contain only colorless urediospores which have shown no signs of germination in hanging drops of water and invariably give rise promptly to prodigious numbers of motile bacteria. Whether these bacteria have any connection with the bleached and apparently parasitized uredia remains to be determined.

Considering the extensive life-history studies that have been published on other Rubus rusts and on rusts in general, it is surprising that so little fundamental research has been conducted on *Kuehneola uredinis*. We have no reference to any such studies of this rust in the United States and only one in Europe. It would seem that this cane rust of blackberries merits some careful study in various parts of the United States. This would include life history and host specialization, which may vary in such widely divergent climates as Washington, southern California, Louisiana, and Maine. Besides the circumstantial evidence given here, additional indication of host specialization is found in the fact that Weiss (6) records the rust on raspberries elsewhere in the United States, while we have not been able to find it on any of the extensively cultivated raspberries or on the wild black species in western Washington.

Specimens of *Kuehneola uredinis* in various spore stages and from various hosts have been deposited in the herbarium of the Department of Plant Pathology, State College of Washington.[3]

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AND
STATE COLLEGE OF WASHINGTON

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[3] Uredia and telia on *Rubus macropetalus*, No. 19876; uredia on *R. macropetalus*, No. 19879; uredia on *R. laciniatus* (cutleaf form), No. 17423; uredia and telia on *R. laciniatus* (cutleaf form), No. 19895; pycnia and aecia on *R. laciniatus* (broadleaf form), No. 19878; pycnia and aecia on *R. laciniatus* (cutleaf form), No. 19877.

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ETHYLENE, A METABOLIC PRODUCT OF DISEASED OR INJURED PLANTS

C. E. WILLIAMSON

(Accepted for publication September 23, 1949)

Ethylene has long been known to cause a wide range of marked physiological responses in plants. The most commonly recognized responses are epinasty, yellow coloration and abscission of leaves, and abscission of flowers and fruits. Ethylene is produced by plant tissues and has been identified chemically as a constituent of the volatiles from ripening fruits (2). The gas in minute amounts is probably a normal metabolic product of certain healthy tissues (1). In one report ethylene in a somewhat increased quantity was identified biologically as a metabolic product of diseased fruits (4).

The rapid yellow coloration and early abscission of leaves infected by certain pathogens suggested the action of a substance such as ethylene. Two diseases characterized by such symptoms are the black spot of rose and the shot hole of cherry caused, respectively, by *Diplocarpon rosae* Wolf and *Coccomyces hiemalis* Higgins. The present study was undertaken to determine whether diseased tissues produced ethylene and, if so, to correlate the symptoms of the disease with the quantity of ethylene produced.

EXPERIMENTAL WORK

The triple response of etiolated Alaska pea seedlings, prepared and grown under standard conditions (5), was used to determine whether or not plant materials produced a physiologically active emanation. The triple response has been defined (3) as a change of negative geotropism to diageotropism, increased growth in thickness, and reduced rate of growth in length. Comparable lots of diseased and healthy tissues were enclosed separately with 35 uniform, etiolated pea seedlings under inverted 3-liter battery jars sealed to glass plates with petroleum jelly. The carbon dioxide produced was absorbed in 30 ml. of 2 N potassium hydroxide solution placed under each battery jar. The experiments were conducted in the dark at 25° C. Each treatment was made in duplicate and each experiment was repeated at least twice. Observations were recorded after 72 hr.

Different qualities of ethylene were produced by the diseased tissues, the amount apparently depending upon the pathogen involved. Large quantities of ethylene were produced by rose leaves infected with *Diplocarpon rosae*, cherry leaves infected with *Coccomyces hiemalis*, and chrysanthemum flowers infected with *Ascochyta chrysanthemi* Stevens (Table 1). Somewhat less ethylene was produced by chrysanthemum flowers infected with *Botrytis cinerea* Pers. ex Fr. and rose leaves infected with *Cryptosporella umbrina* (Jenkins) Jenkins and Wehm. A still smaller quantity, though significantly larger than that produced by healthy tissue, was pro-

duced by rose leaves infected with *Sphaceloma rosarum* (Pass.) Jenkins, oak leaves infected with *Taphrina caerulescens* (Desm. and Mont.) Tul., and carnation foliage infected with *Alternaria dianthi* Stevens and Hall. In the other diseases tested, the infected tissue produced little more ethylene than did the comparable healthy tissue. Bean leaves infected with the halo-blight organism, *Pseudomonas phaseolicola* (Burkh.) Dowson, did not produce ethylene in a detectable quantity.

Healthy leaves in general produce small quantities of ethylene, the amount depending to some extent upon the species of plant (Table 1).

TABLE 1.—*The response of etiolated Alaska pea seedlings exposed to emanations of diseased and healthy plant materials for 72 hr. at 25° C.*

Pathogen	Suscept	Response rating[a] to tissue	
		Diseased	Healthy
Diplocarpon rosae	Rose	5	1
Cryptosporella umbrina	do	4	1
Sphaceloma rosarum	do	3	1
Sphaerotheca pannosa var. *rosae*	do	1	1
Phragmidium mucronatum	do	2	1
Coccomyces hiemalis	Cherry	5	3
Puccinia antirrhini	Snapdragon	2	1
P. chrysanthemi	Chrysanthemum	2	1
Septoria obesa	do	2	1
Ascochyta chrysanthemi	do (flowers)	5	1
Botrytis cinerea	do (flowers)	4	1
Alternaria dianthi	Carnation	3	1
Taphrina caerulescens	Oak	3	1
Phyllosticta minima	Maple	3	1
P. viticola	Ivy	2	1
P. violae	Violet	1	1
Phytophthora infestans	Potato (Green Mountain)	2	1
Rhizopus nigricans	Pea seedlings	1	0
Pseudomonas phaseolicola	Bean (Red Kidney)	0	0

[a] Response of pea seedlings rated from 0 through 5. A rating of 5 indicates no elongation of the seedling, only a clublike knobbing of the growing point. Rating given is that of the majority of four or more trials. Variations of one unit occurred in a few replications.

Shredded healthy rose leaves (Fig. 1) and shredded healthy cherry leaves (Fig. 2) produce considerably more ethylene than do uninjured healthy leaves. Other types of injury apparently may stimulate ethylene production. For example, rose leaves heavily infested with red spider mite, *Tetranychus telarius* L., produced appreciably more ethylene than did healthy rose leaves.

The relation of the condition of the infected tissue to ethylene production was determined for the black-spot disease of rose. Black-spotted rose leaves that had slight yellow coloration, leaves with almost complete yellow coloration, and abscised leaves that had become completely brown but not dry were compared with healthy rose leaves. Ethylene production was most

abundant while the leaf was green, decreased as the leaf became yellow, and ceased when the leaf died and became brown.

The pathogens involved in the diseases tested conceivably could be producing the ethylene and might produce ethylene when growing on an artificial culture medium. Accordingly, recent isolates of *Diplocarpon rosae*,

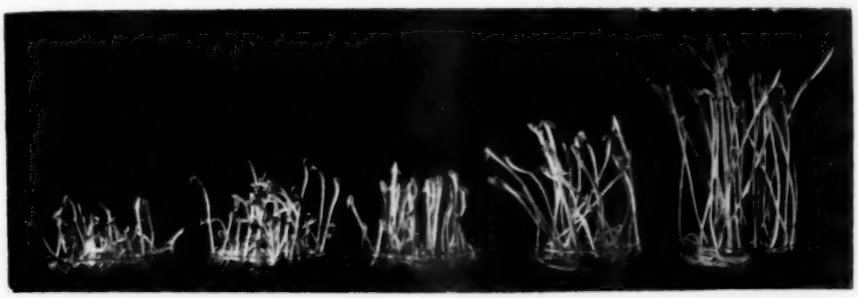

FIG. 1. *Diplocarpon rosae*. Response of etiolated Alaska pea seedlings when exposed for 72 hr. at 25° C. to (left to right): 20 gm. black-spotted rose leaves; 5 gm. black-spotted rose leaves; 15 gm. shredded healthy rose leaves; 15 gm. uninjured healthy rose leaves; no tissue.

Ascochyta chrysanthemi, *Alternaria zinniae* Pape, *Cryptostictis caudata* (Preuss) Sacc., and *Penicillium digitatum* Sacc. were grown on 20 ml. of potato-dextrose agar in Petri dishes. Each test was conducted in quadruplicate on three Petri dish cultures of each fungus. Of the five fungi tested, only *Penicillium digitatum* produced an active emanation.

FIG. 2. *Coccomyces hiemalis*. Response of etiolated Alaska pea seedlings when exposed for 72 hr. at 25° C. to (left to right): 5 gm. black-spotted rose leaves; 5 gm. Coccomyces-infected cherry leaves; 15 gm. shredded healthy cherry leaves; 15 gm. uninjured healthy cherry leaves; no tissue.

The experimental data obtained indicate that for the plant species used in these tests ethylene is produced by the living plant tissue as a response to injury and that the injury produced by certain pathogenic fungi is sufficient to induce ethylene production.

SUMMARY

Ethylene production by diseased and healthy tissues of 12 species of plants was determined and measured biologically by the triple response

of etiolated pea seedlings. Nineteen pathogens were investigated for the ability to stimulate ethylene production.

Certain diseased tissues show a marked stimulation of ethylene production over comparable healthy tissues. The increased evolution of ethylene is apparently a response to injury and occurs only as long as the infected tissue is alive.

Five pathogens were grown on potato-dextrose agar. Only *Penicillium digitatum* produced ethylene from this medium.

The rapid yellow coloration and early abscission of infected leaves seems to be correlated with the production of large quantities of ethylene by the diseased leaves in the case of the black spot of rose and the shot hole of cherry.

CORNELL UNIVERSITY
ITHACA, NEW YORK

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CONTROL OF FUSARIUM STORAGE ROT OF GLADIOLUS CORMS

H. N. Miller and R. O. Magie[1]

(Accepted for publication October 20, 1949)

Heavy corm losses from Fusarium rot in susceptible gladiolus varieties occur during storage. This disease appears to be increasing in Florida; during the 1948–1949 season many growers reported losses ranging from 30 to 90 per cent of the highly susceptible Picardy corms. The storage rot phase of Fusarium corm rot and "yellows disease" causes more loss than is incurred from the disease in the field.

The previous recommendation for control of the disease was treatment of the corms in New Improved Ceresan solution just before planting.[2] At best, this has controlled only rot in the field and not the rotting that occurs during curing and storage of the corms. Growers have tried to prevent storage rot by treating the corms before putting them in cold storage, but results have not been satisfactory. Furthermore, some of the materials used, especially the mercury fungicides, have caused injury to the corms.

From a study of the fungus (*Fusarium oxysporum* f. *gladioli* Snyder and Hansen) and the time of infection, it appears that fungicide protection should be given at the time the corms are cleaned, since much of the infection resulting in storage rot occurs at this time. Gladiolus corms dug from a field having the Fusarium organism in the soil often harbor spores and mycelium of the fungus in roots, trash, and old corms adhering to the new ones. Cleaning the corms exposes a fresh basal plate to the spores of the fungus, an ideal place for infection to occur. This is the region of the corm where many storage rots are initiated. Other infections appear on bruises and injuries incurred during handling operations. Experiments were conducted at the Vegetable Crops Laboratory, Bradenton, Florida, through the 1948 and 1949 seasons to determine the comparative effectiveness of certain fungicides applied to freshly cleaned corms in controlling storage rot.[3]

MATERIALS AND METHODS

Picardy corms, dug from Fusarium-infested fields and known to carry the fungus, were used throughout these tests. They were placed in trays after digging and dried in the field or in a shed for approximately one month or until they could be cleaned easily. The corms were cleaned in a gladiolus shed. Immediately upon cleaning, the bases of the new corms were pressed into a fungicidal dust mixture, or the corms were dipped in

[1] Associate Pathologist, and Pathologist, respectively, Florida Agricultural Experiment Stations.

[2] Creager, D. B. Treating gladiolus bulbs. Florists' Rev. 97 (2517): 39–40. 1946.

[3] Magie, R. O., and H. N. Miller. A new method of controlling storage rot of gladiolus. Florists' Rev. 103 (2655): 35. 1948.

a suspension of the fungicide for 3 to 5 min. Untreated lots of corms were included in each test.

Of the compounds investigated, Fermate (ferric dimethyl dithiocarbamate) plus Dow 9B (zinc trichlorophenate), Parzate (zinc ethylene bisdithiocarbamate) plus Tersan (tetramethyl thiuramdisulfide), thiourea, and New Improved Ceresan (ethyl mercuric phosphate) were used as suspensions. Arasan (tetramethyl thiuramdisulfide) plus Parzate, Fermate plus Dow 9B, Spergon (tetrachloro para benzoquinone), and Seedox (2,4,5-trichlorophenyl acetate) were applied as dusts. The concentrations at which the materials were used are given in the tables.

Just before the corms were placed in cold storage, approximately 3 weeks after cleaning, they were carefully sorted and the diseased and healthy corms were counted. They were again sorted and counted when taken from storage just before planting.

To further determine the effectiveness of the cleaning treatments in controlling Fusarium rots, the corms were planted in the field in factorially designed experiments. In one series of tests the corms were divided into two equal lots. Half of the corms from each of the treatments applied at the time of cleaning were treated with New Improved Ceresan just before planting. The other half were not treated again. In other tests the corms were treated at the time of cleaning and again at the time of planting with the same materials. Data were obtained on number and grade of flowers cut and number and weight of healthy corms harvested.

EXPERIMENTAL RESULTS

Total counts of the corms rotted during curing and storing showed almost twice as many healthy corms in those lots receiving a fungicidal treatment at the time of cleaning as in the untreated lots (Table 1). The

TABLE 1.—*Effect of treating gladiolus corms at the time of cleaning on the incidence of Fusarium rot during the storage period*

Treatments[a]	No. of infected corms	Percentage of healthy corms
Fermate 1/2 per cent + Dow 9B 3/8 per cent (dip)	26	93.5
Parzate 1/2 per cent + Tersan 1/2 per cent (dip)	74	81.5
Arasan 20 parts + Parzate 10 parts + pyrophyllite 70 parts (dust)	50	87.5
Fermate 10 parts + Dow 9B 10 parts + pyrophyllite 80 parts (dust)	39	90.2
Spergon 50 parts + pyrophyllite 50 parts (dust)	31	92.2
Seedox 50 parts + pyrophyllite 50 parts (dust)	47	88.2
Thiourea 1/4 per cent (dip)	227	43.2
Check—no treatment	208	48.0

[a] Each treatment was applied to 400 large Picardy corms.

thiourea treatment was an exception, however. Possibly because of injury, the percentage of healthy corms in this treatment was lower than that for the untreated lot. The Spergon and the Fermate plus Dow 9B treatments

were very effective in controlling storage rot. There were no significant differences in control between the dip and the dust applications of the materials. Seedox and the Dow 9B mixture, especially at higher concentrations, tended to injure the corm surfaces.

Data on flower and corm production showed that all corm lots treated at the time of cleaning with the materials listed in table 1, with the exception of thiourea, gave higher yields than the untreated corm lots (Table 2).

TABLE 2.—*Effect of corm treatments for control of Fusarium on the yield of gladiolus flowers and corms*

Treatment at time of cleaning	Flower production index			Weight of corms produced			Number of corms produced		
	Time of treatment[a]			Time of treatment[a]			Time of treatment[a]		
	1	2	Sum	1	2	Sum	1	2	Sum
Fermate 1/2 per cent + Dow 9B 3/8 per cent (dip)	381[b]	370[b]	751	165[b]	133[b]	298	111[b]	98[b]	209
Parzate 1/2 per cent + Tersan 1/2 per cent (dip)	303	327	630	128	125	253	86	90	176
Thiourea 1/4 per cent (dip)	129	270	399	42	124	166	33	96	129
Arasan 20 parts + Parzate 10 parts + pyrophyllite 70 parts (dust)	399	354	753	135	121	256	96	106	202
Fermate 10 parts + Dow 9B 10 parts + pyrophyllite 80 parts (dust)	353	384	737	130	152	282	90	108	198
Spergon 50 parts + pyrophyllite 50 parts (dust)	380	334	714	150	148	298	105	113	218
Seedox 60 parts + pyrophyllite 50 parts (dust)	238	341	579	95	153	248	64	109	173
Check—no treatment	136	384	520	30	148	178	27	105	132
Sum	2319	2764		875	1104		612	825	
Difference required between sums for significance at odds of 19: 1	192.77		96.38	78.15		30.07	50.65		29.75

[a] Time of treatment 1, corms treated at time of cleaning only. Time of treatment 2, corms treated at time of cleaning with the material listed and again treated with 1/4 per cent New Improved Ceresan dip just before planting.

[b] Differences required for significance in the interaction of material with time of treatment: for flower production index, 68.14; for weight of corms, 27.61; for number of corms, 21.11.

The total yields from the corms treated at the time of cleaning with the materials listed, and again at the time of planting with New Improved Ceresan, were significantly higher than the total yields from corms treated only at the time of cleaning. However, the yields from some of the better treatments, namely, Spergon, the Fermate plus Dow 9B, and the Arasan plus Parzate mixtures, were not increased by the addition of New Improved Ceresan at the time of planting. Spergon used at the time of cleaning and again at the time of planting gave yields as high as any other combination of treatments.

SUMMARY

The treatment of gladiolus corms with a fungicide at the time of cleaning appears to be an important supplementary measure in the control of Fusarium yellows and corm rot in Florida. This control measure may not take the place of the preplanting treatment, but it will make possible the use of a milder preplanting treatment. Materials can be used which are less injurious to the corms, and more effective control can be obtained.

The fungicides which controlled rotting in storage also checked the growth of the fungus in established lesions. It appears that the effective materials not only prevented new infections from occurring, but killed the fungus in some surface lesions. For maximum control it is imperative that the fungicide be applied as soon as feasible after the corms are cleaned.

Spergon used as a 2 per cent dip or 50 per cent dust, and a dip of Fermate $\frac{1}{2}$ per cent plus Dow 9B $\frac{3}{8}$ per cent, were the most effective treatments in controlling the rot. There was no significant difference in control between the dust and dip applications of the fungicides.

The most effective control of Fusarium rot, both in storage and in the field, was a treatment at the time the corms were cleaned with one of the materials listed above, followed by an additional treatment at the time of planting with Spergon used as a 2 per cent dip or with a $\frac{1}{4}$ per cent dip of New Improved Ceresan.

VEGETABLE CROPS LABORATORY
AGRICULTURAL EXPERIMENT STATIONS, UNIVERSITY OF FLORIDA
BRADENTON, FLORIDA

PHYTOPATHOLOGICAL NOTES

Perennation in Relation to Control of Dodders on Native Shrubs in Southern California. KENNETH F. BAKER. Large orange mats of dodder (principally *Cuscuta subinclusa* Dur. & Hilg. and *C. californica* Choisy) are conspicuous features of native vegetation on the dry foothills of southern California by early summer. These parasites frequently become damaging in gardens that include naturalized wild shrubs, growing from them onto cultivated plants. Dodder also commonly invades gardens from adjacent vacant lots. The many requests for information each year prompted this brief study of the problem.

It is commonly assumed that these dodders survive from year to year as seeds, but examination of many plants over several seasons in the West Los Angeles area has failed to demonstrate seed production. The cause of this condition is not known. Bennett[1] reported the same situation for these dodders in the Riverside area and in greenhouses. Dr. Harlan Lewis found that specimens in the Herbarium, Department of Botany, University of California, Los Angeles, had no seeds, and reports that this is the common condition for them in southern California. Mr. Reid Moran examined the specimens in the Herbarium, Department of Botany, University of California, Berkeley. Of 37 collections of *Cuscuta californica* made in or south of Santa Barbara County, only 7 had seeds; of 36 specimens from north of that county, 18 had seeds. For *C. subinclusa* the figures were 7 out of 36 and 9 out of 28, respectively.

Apparently these dodders survive the winter in southern California almost entirely in the vegetative condition. While most of the twining stems die during that period, a very few of the constricting loops survive and sprout in the spring, producing slender orange branches (Fig. 1). On a given host stem, usually only a single circlet remains alive. The total number of such surviving foci of spread in each infested shrub appears not to be more than three or four, and commonly only one. Incidentally, such vegetative propagation favors the persistence of Bennett's[1] latent virus of dodder under natural conditions.

Similar estivation has been noted by Stewart and French[2] for *Cuscuta epithymum* Murr. on the crowns of alfalfa, red clover, and certain weeds in New York, and was considered to be the chief method of survival there. The literature cited in their paper indicated that such survival had been known since 1868 through even the most severe winters in Germany. It was not unusual, therefore, that the California dodders survived about as well as in warmer years the dry, cold winter of 1948–49, during which temperatures reached 23° F. in West Los Angeles.

[1] Bennett, C. W. Latent virus of dodder and its effect on sugar beet and other plants. Phytopath. 34: 77–91. 1944.

[2] Stewart, F. C., and G. T. French. The perennation of the clover dodder, *Cuscuta epithymum* Murr. Torreya 9: 28–30. 1909.

The fact that winter survival of *Cuscuta subinclusa* and *C. californica* in southern California depends on so few haustorial loops suggested a simple means for control in gardens. Removal by pruning of the few perennation foci as they resume growth in the spring, and before secondary infestation occurs, has proved effective and easy. The time for resumption of growth by the dodder circlets varies somewhat in different years and areas, but

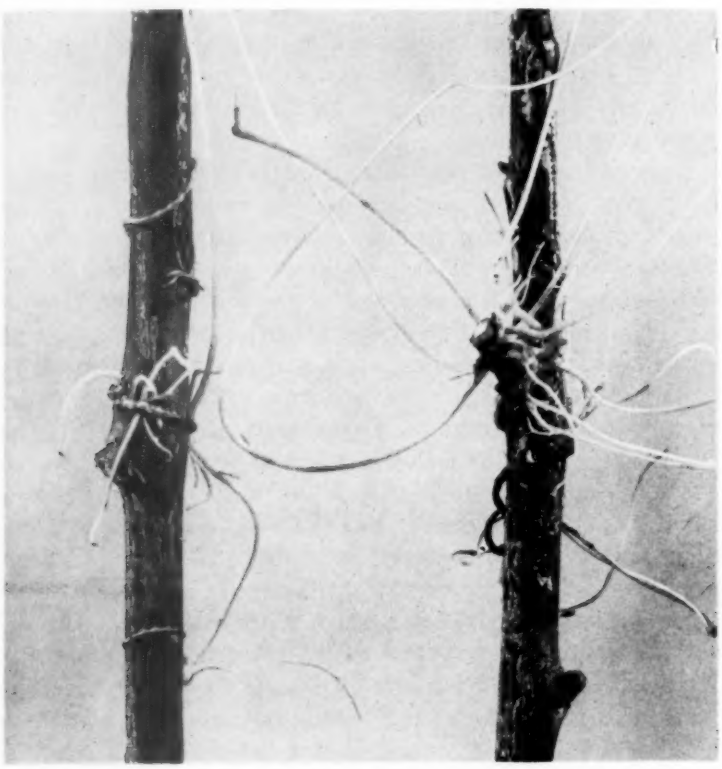

FIG. 1. Perennation of *Cuscuta subinclusa* Dur. & Hilg. on stems of *Rhus laurina* Nutt. Collected March 28, 1946, in West Los Angeles. A few over-wintered loops are shown, from which new tendrils have formed.

generally occurs during March or early April. The stage for ready detection and removal is shown in figure 1. Because the surviving loops in a given location start growth at slightly different times it would be desirable to inspect the plants and remove infested host branches at weekly intervals during the period indicated. Once the dodders have been eliminated from a garden in this way there is little likelihood of reinfestation.—Division of Plant Pathology, University of California, Los Angeles, California.

On the Physical Properties of Sugar-Cane Mosaic Virus. JOSÉ ADSUAR. Sugar-cane mosaic is widely spread in many of the cane-growing countries,

in some of which it has, at one time or another, seriously impaired the sugar-cane industry. Although considerable progress has been accomplished in the control of the disease by the use of resistant varieties, there is still very little information in regard to the physical properties of the virus.

Rafay[1] presents data on aging, dilution end point, action of chemicals, and filtration. Smith[2] includes Rafay's data, and in addition mentions a thermal inactivation temperature without mentioning the source of information. Holmes[3] cites a thermal inactivation point from 53° to 54° C. in 10 min. in leaf tissue.

In view of the meagerness of the data concerning the physical properties of the sugar-cane mosaic virus it seemed desirable to reinvestigate Rafay's studies. Properties studied are in table 1.

Leaves of the sugar-cane variety BH 10/12 (*Saccharum officinarum* L.) showing definite symptoms of mosaic were cut in small pieces and passed through a meat grinder, and the macerate was strained through muslin cloth. In some cases leaves were extracted in a Carver laboratory press at 14,000 lb. pressure. The grinder, cylinder, and plunger of the press were cooled before use. Both methods yielded a highly infectious liquid.

Seedlings about 1 month old of the sorghum variety Hegari (*Holcus sorghum* L.) were used as test plants in the experiments. The seed was planted in flats or in 6-in. pots filled with nonsterile garden soil. Sorghum was chosen because of its susceptibility and the ease with which it is grown and handled in the laboratory. The leaves were dusted with carborundum and rubbed with a cotton swab soaked in the infectious liquid.

Longevity of the virus was determined by aging undiluted juice at room temperature (82° F.). Thermal inactivation temperature was determined by treating 1-cc. samples of the undiluted juice in a thermostatically controlled water bath for 10 min.

TABLE 1.—*Summary of the data obtained in the study of the physical properties of the sugar-cane mosaic virus*

Experimenter	Longevity	Dilution end point	Thermal inactivation	Filterability
Rafay	< 2 hr.	1–10	.....	Failed to pass L_3[a] Chamberland filter
Adsuar	< 7 hr.	1–1000	55° C.	Failed to pass Coors P_2

[a] Equivalent to Coors P_3.

[1] Rafay, S. A. Physical properties of sugar-cane mosaic virus. Indian Jour. Agr. Sci. 5: 663–670. 1935.

[2] Smith, K. M. Textbook of Plant Virus Diseases. 615 pp. P. Blakiston's Sons & Co., Philadelphia. 1937.

[3] Holmes, Francis O. Order Virales. The filterable viruses. Suppl. 2 in Bergey's Manual of Determinative Bacteriology. Ed. 6. 1387 pp. Williams and Wilkins Co., Baltimore. 1948. (*Marmor sacchari* H. is discussed on p. 1183.)

Filtration was done by passing pressed juice through Coors porcelain filter cylinders of four different porosities: P_2, P_3, P_5, P_7; porosities increasing in fineness from P_2 to P_7. A loopful of the chromogenic organism *Sarcina lutea* was added to the juice before filtering and the filtrate tested for sterility in nutrient broth and nutrient agar.

Not less than 6 plants were inoculated in each experiment and every experiment was repeated at least six times. Checks for infectiousness of extracted juice were carried out in all tests made. Table 1 shows our results as compared with those of Rafay.—Agricultural Experiment Station, Rio Piedras, Puerto Rico.

Arrested invasion of Lycopersicon peruvianum roots by the root-knot nematode[1]. SHUBERT C. LIAO AND A. A. DUNLAP. The penetration of the seedling roots of the South American tomato, *Lycopersicon peruvianum* (L.) Mill., was studied microscopically in comparison with roots of the common tomato, *L. esculentum* Mill. In greenhouse tests during the winter of 1947–1948, seeds of both species were planted in 6-in. pots containing soil infested with the root-knot nematode (*Heterodera marioni* (Cornu) Goodey). The strain of *L. peruvianum* used was a nematode-resistant selection from P. I. 128651, an accession from the Division of Plant Exploration and Introduction, U. S. Department of Agriculture. The susceptible variety was Earliana. About 2 days after emergence of the seedlings, the cotyledons were well expanded but the first true leaves had not developed. At this stage, the seedlings were carefully removed from the soil by submerging the pot in water and gently dislodging the roots. The taproots were then cut near the soil line and placed in hot acetone.[2] They were then placed in Flemming's stronger fluid for 2 hr. After dehydration in alcohol the roots were cleared in clove oil and studied in this medium.

In the root tips of *L. esculentum*, the nematodes had penetrated and were abundant in the distal portion of the central cylinder (Fig. 1, A). In *L. peruvianum*, however, only a few nematodes had penetrated the root near its tip and these were located for the most part in the peripheral cells (Fig. 1, B). In the figure two or three of the larvae may be seen only partially embedded in root tissue.

In a second experiment, seedlings were grown for 4 weeks before removal from the soil. During this time seedlings of the susceptible species became yellow and died. Only 3 of 40 seedlings of *L. peruvianum* showed typical root-knot swellings. On these there were 12, 4, and 2 small galls per plant, respectively. None of the 40 plants had above-ground symptoms of disease. When the roots of these plants were fixed and examined microscopically, nearly all of the nematodes that had attacked the roots were clustered around

[1] Published with the approval of the Director as Technical Article No. 1218, Texas Agricultural Experiment Station.

[2] Godfrey, G. H. The demonstration of plant-parasitic nematodes in host tissues. Phytopath. 25: 1026–1030. 1935.

the young root buds which were just breaking through the cortex of the main taproot. Few of the larvae had advanced beyond this point. In-

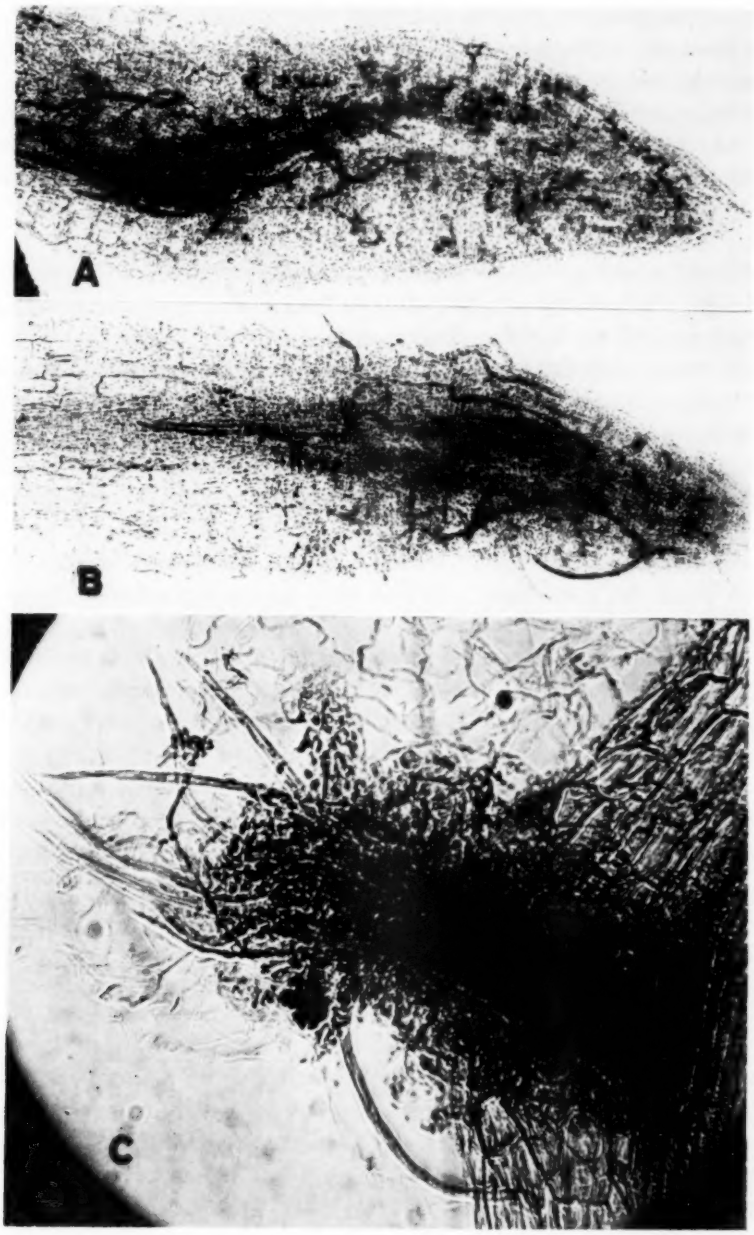

FIG. 1.—A. Root tip of *Lycopersicon esculentum* invaded by many root-knot nematodes 2 days after emergence of the seedling in infested soil. B. Comparable root tip of *L. peruvianum* only partially invaded by the nematodes. C. Lateral root bud of *L. peruvianum* 1 month after emergence showing arrested penetration of several nematode larvae.

vasion of the root tissues was arrested when approximately one-half of the worm had become embedded in the growing point of the young rootlet (Fig. 1, C). Examination of some of the partially infested root buds under the microscope prior to fixation indicated that many of the larvae had died in this position. The roots of *L. peruvianum* offer a striking illustration of nematode resistance. The differences in degree of penetration do not seem to be related to any observable structural differences between root tips of the two tomato species. The presence of a chemical inhibitor in the resistant one is therefore suggested.—Department of Plant Physiology and Pathology, Texas A. & M. College System, College Station, Texas.

Actidione as a Plant Protectant. DAVID GOTTLIEB, H. H. HASSAN, AND M. B. LINN. The efficacy of actidione as a fungistatic agent for many yeasts was first reported by Whiffen, Bohonos, and Emerson.[1] Later Felber and Hamner[2] stated that this material will control powdery mildews, and at the time this paper is being prepared for publication, Vaughn et al.[3] have shown that actidione is toxic to some higher plants. Because of its possible use as a plant protectant this antibiotic has been studied in our laboratory for 1) its fungicidal properties against a representative group of fungi, and 2) its toxicity to some higher plants.

The inhibiting properties of actidione were determined by growing the fungi in Blakeslee's medium to which various concentrations of the antibiotic from 0.1 to 1000 p.p.m. had been added. Disks of the six fungi (*Sclerotinia fructicola, Pythium graminicola, Glomerella cingulata, Fusarium lycopersici, Rhizoctonia solani,* and *Stemphyllium sarcinaeforme*) cut from agar plates were used to infest the liquid medium, and growth was recorded after 7 days. A value of 10 was assigned to the mycelium in the control flask, and the growth in other flasks was rated from 0 to 10 by visual inspection. The results (Table 1) indicate that growth retardation began

TABLE 1.—*Growth of fungi in the presence of actidione*

Organism	Growth[a] at various concentrations, in p.p.m.						
	0	0.1	1	10	50	100	1000
Sclerotinia fructicola	10	10	10	8	4	3	0
Pythium graminicola	10	10	4	3	1	0	0
Glomerella cingulata	10	10	10	5	0	0	0
Fusarium lycopersici	10	10	10	8	3	0	0
Rhizoctonia solani	10	6	4	2	0	0	0
Stemphyllium sarcinaeforme	10	10	1	0	0	0	0

[a] Growth rated on a scale of 1 to 10.

[1] Whiffen, A. J., N. Bohonos, and R. L. Emerson. The production of an antifungal antibiotic by *Streptomyces griseus.* Jour. Bact. **52**: 610–611. 1946.

[2] Felber, I. M., and C. L. Hamner. Control of mildew on bean plants by means of an antibiotic. Bot. Gaz. **110**: 324–325. 1948.

[3] Vaughn, J. R., J. L. Lockwood, G. S. Randwa, and C. Hamner. The action of actidione on plant tissue and upon certain fungi. Michigan Agr. Exp. Sta. Quar. Bul. **31**: 456–464. 1949.

at concentrations between 0.1 and 10 p.p.m. for the different fungi. All the fungi except *S. fructicola* were inhibited by 100 p.p.m.

Five different types of plants (tomato, bean, geranium, peach, and strawberry) were sprayed by means of a hand atomizer with actidione at concentrations of 1, 10, 100, and 1000 p.p.m. Of these plants all but strawberry were injured at 1000 and 100 p.p.m. Tomatoes and beans were most sensitive to this material: at 1000 p.p.m. all the leaves were killed, at 100 p.p.m. about 50 per cent of the leaf area was necrotic, while at 10 and 1 p.p.m. small necrotic spots covered about 15 and 10 per cent of the leaf, respectively. Geranium leaves had approximately 33 per cent of their leaf area burned at 1000 p.p.m. and 10 per cent at 100 p.p.m., with no apparent injury at lower concentrations. Peach twigs were completely defoliated 10 days after the two higher concentrations were applied, but no effect was noticeable with the lower concentrations. The strawberry plants were not sensitive to actidione at any of the concentrations used. These plants, however, were not in an active growth phase at the time of spraying, which might account in part for their tolerance of actidione.

Despite the fungicidal efficacy of actidione, its practical use will depend in part upon its toxicity to the host. Its injurious effects on some plants may be so severe as to preclude its use.—Department of Horticulture, University of Illinois, Urbana, Illinois.

Cross Protection Tests with Cucumber Viruses 3 and 4 and Tobacco Mosaic Virus. ROBERT W. FULTON. It has been shown by Bawden and Pirie[1,2] and Knight and Stanley[3] that cucumber viruses 3 and 4 and ordinary tobacco mosaic virus have a common antigen as well as similar physical and morphological characteristics. Since the host range of cucumber viruses 3 and 4 is limited to the Cucurbitaceae, there appeared to be no host on which relationships between the viruses could be checked by means of a cross protection test. In a recent publication[4] it was shown, however, that the tobacco mosaic virus locally infects cucumber leaves and cotyledons, and that some strains produce distinct lesions. It was apparent that this host could be used to determine whether previous infection with cucumber virus 3 or 4 could prevent infection by tobacco mosaic virus.

The tests were necessarily based on the inoculation of tobacco mosaic virus to cucumbers already infected with either cucumber virus 3 or 4. In most of the trials a strain of tobacco mosaic virus (previously[4] referred to as KC), which produced distinct local lesions on cucumber cotyledons, was used. In the first trials cucumber cotyledons were inoculated with the

[1] Bawden, F. C., and N. W. Pirie. Liquid crystalline preparations of cucumber viruses 3 and 4. Nature **139**: 546. 1937.

[2] Bawden, F. C., and N. W. Pirie. The relationships between liquid crystalline preparations of cucumber viruses 3 and 4 and strains of tobacco mosaic virus. Brit. Jour. Exp. Path. **18**: 275–290. 1937.

[3] Knight, C. A., and W. M. Stanley. Aromatic amino acids in strains of tobacco mosaic virus and in the related cucumber viruses 3 and 4. Jour. Biol. Chem. **141**: 39–49. 1941.

[4] McKinney, H. H., and Robert W. Fulton. Local susceptibility of cotyledons and leaves of cucumber to tobacco mosaic virus. Phytopath. **39**: 806–812. 1949.

cucumber viruses, and after 4 to 6 days inoculated with the tobacco mosaic virus. Local lesions appeared on these cotyledons in approximately the same numbers as on cotyledons which had not been inoculated with the cucumber viruses.

In other tests cucumber leaves systemically infected with cucumber viruses 3 or 4 were inoculated with tobacco mosaic virus. Six to 8 days later these leaves were ground and inoculated to other cucumbers systemically infected with the respective cucumber viruses. After 5 successive transfers on cucumbers systemically infected with cucumber virus 3 or 4, tobacco mosaic virus was readily demonstrable by inoculation to the *Nicotiana tabacum* × *N. glutinosa* hybrid. The cucumber viruses alone produced no symptoms on the hybrid.

The extent of multiplication of tobacco mosaic virus in the presence of cucumber virus 4 was shown by inoculating halves of cucumber leaves with the cucumber virus. The opposite half-leaves were wiped with healthy cucumber juice. Five days after the first inoculation, tobacco mosaic virus was applied to the entire surface of the leaves. Four days later 4-mm. disks were punched from opposite half-leaves with a cork borer. The disks were ground, diluted with pH 7 phosphate buffer at the rate of 2.5 ml. per disk, and inoculated to half-leaves of the hybrid test plant. Samples from cucumber half-leaves previously inoculated with cucumber virus 4 produced an average of 291 lesions per half-leaf of the hybrid test plant. Samples from the opposite halves of the cucumber leaves, not previously inoculated with cucumber virus 4, gave an average of 242 lesions per half-leaf of the hybrid test plant.

These trials failed to demonstrate any protective effect of the cucumber viruses against tobacco mosaic virus. The results need not necessarily be interpreted, however, as indicating a complete lack of relationship between these viruses. It seems possible that the degree of relationship between the cucumber viruses and tobacco mosaic virus may be less than that required to produce a protective or competitive effect.—Division of Tobacco, Medicinal, and Special Crops, U. S. Department of Agriculture, and University of Wisconsin Agricultural Experiment Station, Madison, Wisconsin.